ASSOCIATION OF LEPTIN GENE POLYMORPHISM IN ESSENTIAL HYPERTENSION

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BONAFIDE CERTIFICATE

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CONTENTS

Introduction

INTRODUCTION

Hypertension is an evolving global burden to health. Essential hypertension (EH) is an intricate disorder with various genetic and environmental influences leading to its occurrence. Research on animal models have revealed that polygenic hypertension is based on complex genetic architecture and potent genomic regulations. The contribution of genetic factors to hypertension have also been revealed from the considerable clustering in families and the constancy among twins. Thus by identifiying the genes vulnerable to cause essential hypertens in it'll be useful in defining its pathophysiology and in recognising the high risk populations and to decide on specific therapy regime.

Arterial hypertension is one of the key risks in development of cardiovascular diseases. Despite few recognized uncommon types of secondary hypertension, essential hypertension seems to be the most frequent diagnosis. The heritable basis of this disorder has been well noted in numerous familial studies. The amount of input by the genetic factors to variations in blood pressure is roughly around 30%, yet it remains complex and presently not fully understood. This study is framed to focus on the genetic backdrop of essential hypertension with particular attention to its association with leptin gene .

Epidemiological data propose that 65–75% of possibility for hypertension is ascribed to increased weight[1,2]. Obesity probably via enhanced SNS activity, remains a source for amplified cardiovascular morbidity and mortality. Recently, hormones secreted by adipose tissue are gaining significance in research studies linking obesity and hypertension. Studies have shown that adipose tissue has become an important regulatory site that produces numerous immune-modulators and biologically active molecules[3,4] . Among these diverse substances, leptin seems to have evolved as an imperative hormone with significant pleiotropic activities on multiple organs [5,6].

Leptin , a 16-kDa-peptide is chiefly produced by the adipose tissue. Majority of its functions is via its action on hypothalamus. It controls energy balance by drop in appetite and rise in temperature. Thus it regulates appetite and energy outflow. Also growing research datas substantiate that leptin, via multiple mechanisms, possibly has a significant influence on the regulation of CVS and renal and sympathetic nervous system outflow. Though the importance of endogenous leptin requires more elucidation, physiologically it seems to be involved in pressure and volume-regulation. Conversely, in pathological states like chronic hyperleptinemia as seen in obesity, perhaps it might lead to evolvement of hypertension and probably cause injury to cardiovascular and renal system. Leptin induced sympathetic activation must be the most likely cause for obesity linked hypertension. Even though obesity is

commonly related with opposition to the appetite suppression and weight lowering effecs of leptin, there is conservation of its sympathetic activation and pressor effects. This discriminating effect associated with hyperleptinemia, could be a crucial cause for the cardiovascular manifestations in obesity.

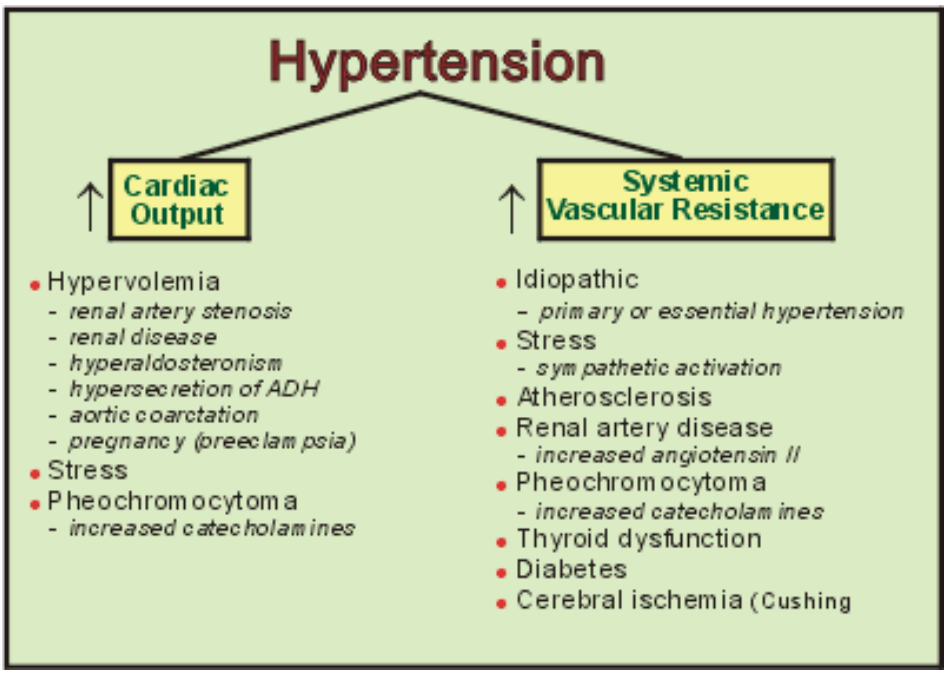
Review of Literature

REVIEW OF LITERATURE

HYPERTENSION:

Hypertension can be classified into two major categories based on its etiology. They are primary or essential hypertension (EH) and secondary hypertension.(Figure:1) Secondary hypertension are rare forms of hypertension with well established causes. Essential hypertension is the most frequent type of hypertension. EH is postulated as a long standing increase in blood pressure with no known, distinct cause[7]. Essential hypertension contributes to significant morbidity and mortality. However frequent, it currently remains as a composite disorder with indefinable causes. Being a continuous variable, BP is a quantitative trait. Latest animal model data revealed a complex genetic structure of quantitative trait loci (QTLs) for blood pressure (BP). From various familial and epidemiological studies it is understood that hypertension occurs due to composite interaction among heritable and environmental factors. Intricate QTL–QTL connections and potent genomic control mechanisms are the basis of polygenic hypertension. Research works are needed further to offer likely genetic mechanisms to describe the basis of essential hypertension.

FIGURE: 1



COURTESY : CVPHYSIOLOGY.COM

FIGURE:2 :

JNC 7TH REPORT- GUIDELINES FOR DIAGNOSIS OF HYPERTENSION

TERMINOLOGY	Systolic BP	Diastolic BP
Normal	≤ 120	≤ 80
PRE HYPERTENSION	120-129	80-84
	130-139	85-89
HYPERTENSION	≥140	≥90
Stage 1 (Mild hypertension)	140-159	90-99
Stage 2 (Moderate hypertension)	160-179	100-109
Stage 3 (Severe hypertension)	≥ 180	≥ 110
Isolated Systolic hypertension	≥ 140	≤ 90

Definition:

According to the recent guidelines from the American Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VII) [7] hypertension is defined as a systolic blood pressure ≥ 140mm Hg and/or a diastolic blood pressure ≥ 90mm Hg. (Figure:2)

The criteria for defining hypertension varies between many research groups. Thus the level of BP beyond which anti-hypertensives will become beneficial than left untreated can be used to define HT.

Guidelines & Classifications:

Different organizations have generated disease-specific guidelines for various conditions and hyper tension is one among them. The JNC VII report is given in figure:2

Aetiology of hypertension:

There are two types:

a. Primary or essential hypertension :

It is a multifaceted disease with obscure causes. The genetic and ecological factors contributing to its prevalence makes it a multifactorial disorder. Over 95% of patients with hypertension present with such a picture.

1. Genetic predisposition – A family history of hypertension, heart disease, type2 diabetes mellitus
2. Non –modifiable factors– Age
3. Modifiable factors – Stress, high calorie diet, overweight, smoking, heavy drinking, sodium rich diet, lack of physical exercise.

b. Secondary hypertension :

2 to 5%; has an underlying cause, usually involving the renal and the endocrine system

Causes:

a) Endocrine :

Adrenocortical hyperfunction –

1. Cushings' disease and syndrome
2. Primary hyperaldosteronism
3. Congenital adrenogenital syndrome (17α-hydroxylase and 11α -hydroxylase defects)
4. Myxedema
5. Acromegaly

6. Adrenal medullary involvement: Pheochromocytoma

b) Vascular :

1. Coarctation of aorta
2. Stenosis of renal artery
3. Renal infarction

c) Renal :

1. Intrinsic renal disease
2. Polycystic kidney
3. Chronic kidney diease

d) Medications :

1. OCPs
2. Adrenergic drugs

Epidemiology :

24% of all coronary heart disease deaths and 57% of all stroke deaths are directly attributed to Hypertension in India[8].

Pathogenesis:

The pathogenesis is multifactorial and highly intricate for essential hypertension. Several factors alter the pressure in the vasculature for adequate blood supply to various organs. They include humoral mediators, cardiac output, circulating volume, viscosity, blood vessel elasticity , vascular calibre,

endothelial response, and neural stimulation. Multiple factors have been proposed as possible pathogenesis of essential hypertension. They include

Heredity:

Genetic factor has been understood to be involved in the genesis of hypertension. Most studies sustain the theory that the genetic basis is most likely multifactorial. It is more likely to have a complex association of multiple genes rather than a single gene to account for the heritability of this disorder. Both susceptibility genes and monogenic defects have been reported to be having one of their consequences an increased arterial pressure. Various epidemiological studies and familial studies imply the complex interplay among various factors such as genetic susceptibility, ecological lifestyle exposures like intake of sodium in food, surplus alcohol consumption and body weight[9]. Also it has been suggested from twin studies that inter-individual variation of BP is heritable [10].

Epigenetic mechanisms:

Aberrant DNA methylation has been linked to many age related disorders including hypertension[11,12] . For example, the epigenetic dysregulation of estrogen receptor β has been found to participate in development of essential hypertension [13]

Intra-uterine factors:

Several data suggest a possibility that the blood pressure of an adult is established depending on the birth weight of a fetus. It is a vicious cycle showing pregnant women with high normal or even greater blood pressure reproduces low birth weight babies. These babies consequently develop high normal blood pressure and in due course they develop hypertension which may be due to the associated metabolic abnormalities such as IR, DM, lipid disorders, and abdominal obesity. This theory is named as "Barker hypothesis". [14]

Environment:

A number of environmental factors including high sodium intake, excess weight gain, alcohol ingestion play an important role in elevation of blood pressure along with age.

PATHOPHYSIOLOGY

Cell membrane defect:

Generalised cell membrane defect is one possible explanation. This hypothesis derives most of its information from the studies on circulating blood elements, which have documented the abnormalities in the sodium transport across the cell membrane. Several cells of the body, in particular, the vascular

smooth muscle cells exhibit this defect. This causes an abnormal calcium accumulation in the vascular smooth muscle which in turn leads to increased vascular reactivity.

Intravascular Volume

The two main determinants of hypertension are raised cardiac output and increased peripheral vascular resistance[15]. Cardiac output is calculated by heart rate and stroke volume. The latter inturn depends on the force of contraction and the ventricular volume. Peripheral resistance depends on anatomic and functional alterations in of the blood vessels.

Vascular volume is calculated by arterial pressure over the long term. Variations in total ECF volume is directly related to the blood volume. Extracellular fluid volume is calculated by sodium which is an extracellular ion. When the intake of sodium chloride exceeds the kidney's capacity to excrete sodium, vascular volume expands and cardiac output increases. However, various vascular beds including brain and kidney have the capacity to self regulate blood flow, and the resistance within that bed must increase, if constant blood flow is to be maintained.

Blood flow = pressure across the vascular bed / vascular resistance[15].

Impaired sodium excretion :

Impaired excretion of sodium leads to water retention which in turns increases the blood volume . Changes in the blood volume can alter the bp. In patients with CKD, blood volume correlates with the systolic blood pressure. This is mediated by the abnormal vasoregulation and an increased systemic vascular resistance[16]. In individuals with an impaired capacity to excrete sodium, greater increases of arterial pressure are required to achieve natriuresis and sodium balance.

Impaired pressure natriuresis:

The effect of sodium on blood pressure is associated to the provision of chloride with sodium. High sodium chloride intake causes increase in arterial pressure, and sodium excretion through urine increases and sodium balance is maintained at the expense of an increase in arterial pressure. The mechanism is called "pressure-natriuresis" phenomenon. In Chronic kidney disease there is impaired pressure natriuresis leading to loss of ability to alter sodium handling based on small changes in bp. Hence the hypertensive response to Angiotensin-II and aldosterone is diminished when the increased pressure is transmitted to the kidney[16].

Autonomic Nervous System

Sympathetic nervous system stimulation can lead onto either constriction or dilatation of the arterioles. Hence the SNS plays a vital function in

maintainence of bp in the physiological range. Blood pressure variations due to physical exercise, stress are also regulated by SNS. The mediators involved in blood pressure regulation are signals based on blood volume, pressor effects and chemoreceptors. Short term regulation of bp is via sympathetic reflexes and the long-term regulation involves the combined effects of SNS, volume and hormone-related factors. Norepinephrine, dopamine, and epinephrine are the three endogenous catecholamines which play an important role in tonic and phasic cardiovascular regulation.

Several reflexes modulate blood pressure. The arterial pressure increases the rate of firing of the baroreceptors in the aortic arch and carotid sinuses, and the effect is a decrease of sympathetic outflow, resulting in decreases of heart rate and arterial pressure. This is a primary method for quick buffering of sharp fluctuations of arterial pressure that may occur during behavioral or physiologic stress, postural changes, and changes in blood volume. Patients with autonomic neuropathy and impaired baroreflex function may have extremely labile blood pressures with difficult-to-control episodic blood pressure spikes. Medications interfering with the SNS act as potent antihypertensive agents, representing the main part played by SNS in the maintenance of increased arterial pressure.

RAAS:

The renin-angiotensin-aldosterone system is the most important pathway in the maintenance of blood pressure through the sodium-retaining properties of aldosterone and the vasoconstrictor properties of angiotensin II [17].

Increased activity of the renin-angiotensin-aldosterone axis is not invariably associated with hypertension[18]. In response to a low-sodium chloride diet or to volume contraction, arterial pressure and volume homeostasis may be maintained by increased stimulation of the RAAS.

Vascular Mechanisms

Blood pressure is mainly determined by the vascular radius and compliance of resistance arteries. Flow resistance varies inversely with the radius (r^4), and consequently smaller the lumen size more is the resistance. The lumen diameter of small arteries and arterioles of hypertensive patients may reduce with structural, mechanical, or functional changes. In subjects with raised bp the arteries become stiffer, and those with arteriosclerosis exhibit mostly high SBP and wide pulse pressures as a result of reduced arterial compliance due to structural changes in the vascular wall. Vascular endothelial function also modulates vascular tone by releasing vasoactive substances[19-21]. The nitric oxide is a potent vasodilator synthesized by the vascular endothelium. Endothelium-dependent vasodilation is impaired in hypertensive patient.

Insulin resistance:

The increased arterial pressure has been attributed to Insulin resistance and hyper insulinaemia. The possible mechanisms that have been postulated are as follows[22].

a. Insulin has mitogenic action, which increases vascular smooth muscle hypertrophy.

b. It causes retention of renal sodium and increased sympathetic activity, thereby increased arterial pressure.

c. Ion transport across the cell membrane is increased by Insulin, increasing the cytosolic calcium levels of insulin-sensitive renal or vascular tissues.

Recently studies have demonstrated that high blood pressure is associated with high plasma leptin levels. An association between leptin and hypertension has been established clearly from data of available animal studies, whereas results of human studies are less consistent. There are several possible mechanisms, which include

a. One possible explanation is through sympathetic activation. Leptin administered intravenously and intracerebroventricularly in rodents has

been found to increase the sympathetic outflow to kidneys, skeletal muscle vasculature and the neural traffic to the adrenal[23,24]

b. It induces endothelin-1, which is a potent vasoconstrictor and mitogen[25]

c. It promotes angiogenesis, which contributes to the modulation of endothelial cell proliferation in atherosclerosis[26].

d. It increases sodium and water excretion via a direct tubular action. But during leptin resistance it produces anti-natriuresis leading to hypertension[27].

Pathologic Consequences of hypertension :

All clinical manifestations of atherosclerosis have hypertension as a possible etiological role. CAD, heart failure, retinopathy, kidney disorders, peripheral arterial disease (PAD) and stroke have hypertension as an independent predisposing factor.

Heart

The most frequent cause of death in hypertensives is due to cardiac involvement [28]. Cardiac disease due to hypertension is the consequence of functional and structural adjustments resulting in diastolic dysfunction, LVH,

heart failure due to congestion, alterations in the flow of blood, CAD , microangiopathy and arrhythmias.

Vigorous control of hypertension results in reversal of LVH and reduction in the possibility of development of CVD [29,30]. In hypertensive patients, diastolic dysfunctions are frequent and remain as the premature outcome of hypertension. They vary from asymptomatic cardiac involvement to evident cardiac failure. In cardiac failure due to diastolic dysfunction the ejection fraction is conserved as it results from systolic function.

CNS

Brain infarction and hemorrhage encompass hypertension as an important risk factor[31-33]. Stroke is also due to infarction and hemorrhage, either intracerebral hemorrhage or subarachnoid hemorrhage, thus linked to hypertension[34]. The rise in bp, predominantly the SBP in elderly increases progressively the incidence of stroke. The occurrence of stroke convincingly decreases with treatment of hypertension. Hypertension is linked with impaired cognition in old age people. Autoregulation of cerebral circulation[35,36] fails, resulting in vasodilation and hyperperfusion ultimately leading to encephalopathy In malignant hypertension patients.

Kidney

Primary kidney disorder is the most frequent source of secondary hypertension. On the other hand, renal injury and ESRD are caused by hypertension. The increased risk linked with high blood pressure is graded, continuous, and present throughout the entire distribution of blood pressure above optimal[37-43]. The preglomerular arterioles are primarily affected by the hypertension-related vascular lesions in the kidney [44], leading to ischemia of the glomeruli and postglomerular structures. There is hyperperfusion in the glomerular capillaries leading to local injury and finally progressing to glomerulosclerosis[45], ultimately there is resultant ischemia and gradual atrophy of the renal tubules.

Peripheral Arteries

Blood vessels contribute to the pathogenesis of hypertension , and as a result become a target organ for atherosclerotic disease. Hypertensive patients with arterio occlusive diseases of the lower limbs have greater chance of acquiring CVD at a later stage. Several studies suggest that an ankle-brachial index < 0.80 is associated with elevated blood pressure, particularly systolic blood pressure. Patients with hypertension are three times more prone to peripheral vascular disease as apparent by intermittent claudication. In larger vessels like the aorta, it progresses to aneurysm.

Retinopathy

Hypertensive retinopathy is caused by the vascular changes in the eye induced by hypertension[46]. The increase in bp causes a series of pathophysiological changes in the retinal circulation[47]. Keith, Wagener and Barker have classified these vascular changes into four grades. Malignant hypertension, the most severe form, is clinically defined as elevated bp combined with one or more of the following:

1. Bilateral retinal flame-shaped haemorrhages
2. Cotton wool spots
3. Hard exudates
4. Papilloedema.

Diagnosis:

The key to diagnosis of hypertension is the precise measurement of the blood pressure. The preferable mode of recording blood pressure is by using a mercury manometer and taking average of three reading taken two minutes apart. Supine and sitting positions blood pressure measurements should be recorded, auscultating with the bell of the stethoscope. A wider cuff is preferred as even the size of the cuff may affect the blood pressure measurement. The subject should relax calmly for minimum five minutes prior to the recording of BP. Palpation of all peripheral pulses should be performed.

Laboratory studies:

The following routine laboratory investigations should be carried out in all cases of hypertension to make out the probability of target organ injury and to present a prior data to monitor the side effects of the treatment.

* Urinalysis
* Hematocrit
* Serum potassium
* Plasma glucose
* Calcium
* Serum creatinine
* Electrocardiogram (ECG)
* Chest radiography
* Fasting plasma cholesterol and triglyceride levels for hyperlipidemia
* Uric acid
* Fasting plasma cholesterol and triglyceride levels for hyperlipidemia
* Echocardiography in selected cases to monitor the cardiac status and to identify LVH

When a secondary cause is suspected or when the bp is not controllable, extensive investigations have to be carried out.

Treatment:

Lifestyle modifications – JNC VII recommend the following to lower blood pressure and decrease the risk of cardiovascular disease[48-51].

- Weight reduction[52-54].
- Limiting alcohol intake and smoking[55,56].
- Increased physical activity[57].
- Sodium intake reduction to less than 6 gms[58,59].
- DASH – dietary approaches to stop hypertension with adequate dietary potassium, calcium & magnesium[59].
- Reduction of intake of dietary cholesterol and saturated fats.

The JNC VII report[60] recommends the following Initial therapy:-

* Pre-hypertension (SBP – 120-139 mm Hg, DBP – 80-89 mm Hg): anti-hypertensive drug is not indicated

* Stage I (SBP – 140-159 mm Hg, DBP – 90-99 mm Hg): Thiazide diuretics are recommended. beta blocker, ACE inhibitor, CCBs or a combination of these may be used.

* Stage II (SBP – 160 mm Hg, DBP – 100 mm Hg): Two drug combination (Thiazide diuretic and ACE inhibitor / beta blocker) is recommended.

Prevention:

Prevention, early detection and ample treatment forms the broad strategy for reduction in morbidity and mortality from hypertension.

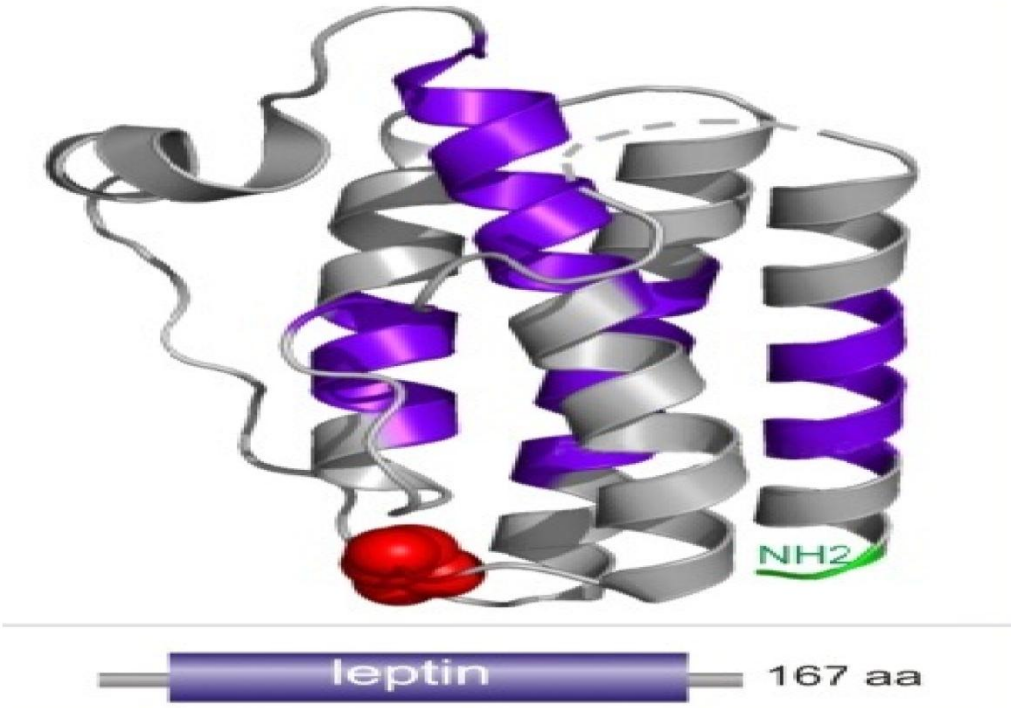
Even a minimum decline in BP imparts considerable improvement in health. There is a considerable drop off in the occurrence of stroke (35%) & CAD (16%)[61] by a fall in SBP by 10-12 mmHg and DBP by 5 -6 mm Hg.

Prevention includes

- Control of weight
- Increase in physical activity
- Moderate salt intake
- Increased intake of K+
- Reduced ingestion of alcohol
- Regular diet comprising adequate quantity of the following
- - fruits
- -vegetables
- - low fat meat
- - fish
- -dairy products

LEPTIN:

FIGURE – 3



Discovery:

Leptin is an adipocyte derived hormone[62]. It was discovered by Jeffrey M. Friedman & co - Rockfeller University in the year 1994 [63]. It was named so from the greek word leptos- meaning thin. The gene coding for leptin is Ob gene. It is located on chromosome 7q31.3 in humans[64,65].

Structure:

The ob gene produces a 167 aminoacids containing protein. (Figure:3) Its signal peptide sequence at the amino terminal contains 21 aminoacids. This

secretory signal sequence results in the transport of leptin into microsomes. It is subsequently cleaved off resulting in circulating leptin with 146 aminoacids[63]. Leptin is a 16 kDa peptide. It is a globular protein with a tertiary structure similar to hemopoietic cytokines such as interleukin and granulocyte macrophage colony stimulating factor[66].

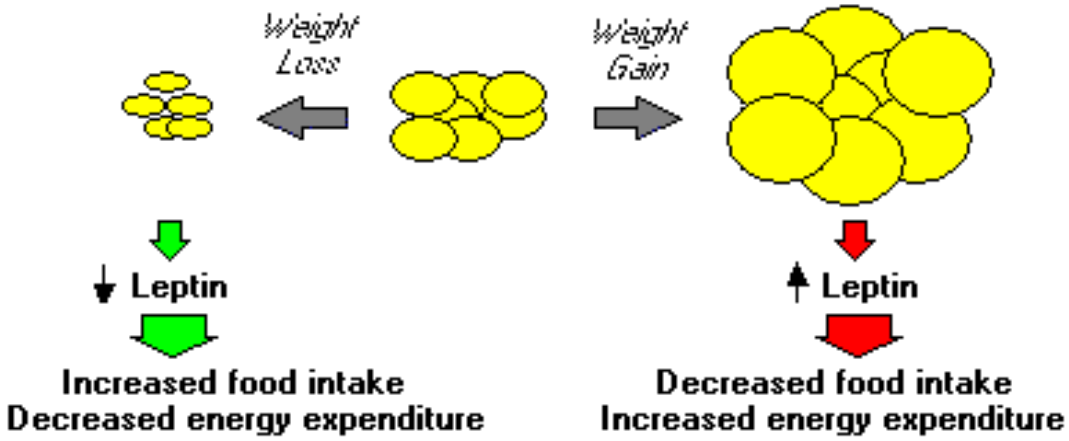
Sources :

The major source of leptin is white adipose tissue. It is also synthesised by brown adipose tissue and other extra adipocyte tissues such as brain, pituitary, placenta (syncytiotrophoblasts), ovaries, mammary epithelial cells[67], skeletal muscle, bone marrow[68], gastric chief cells, lower part of fundic glands, P/D1 cells in the stomach and liver[69,70]. Its secretion is constitutive. There is no storage form for leptin[71].

Functions :

Leptin circulates in proportion to the adipose tissue present in human body . (Figure:4) Initially soon after its discovery leptin was well known for its satiety action. This was accompolished via its action over hypothalamus mediating appetite suppression, energy expenditure, acceleration of metabolic rate and thermogenesis[72,73] thereby controlling the body weight and regulating the accumulation of fat. (Figure:5) Recent studies suggest that leptin has a wide range of functions involving various systems like the renal,

FIGURE - 4


Weight Loss
Weight Gain
Leptin
Leptin
Increased food intake
Decreased energy expenditure
Decreased food intake
Increased energy expenditure

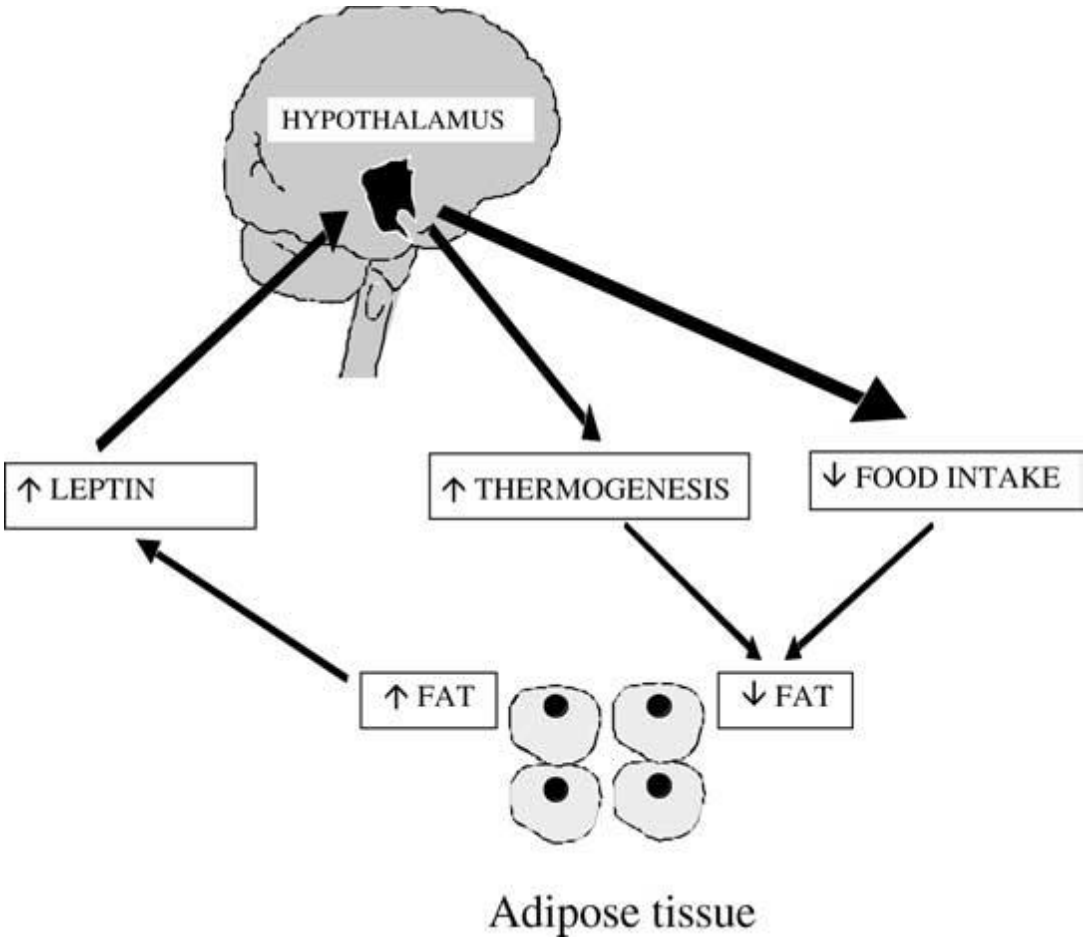


FIGURE 5. Role of leptin in regulation of adipose tissue mass leptin is secreted by adipocytes and circulates in the blood in concentrations proportional to fat mass content. interaction of leptin with its receptor in the hypothalamus inhibits food intake and increases energy expenditure through stimulation of sympathetic nerve activity. this leads to a reduction in adipose tissue mass.

cardiaovascular and sympathetic nervous system [5,6,74-77]. Its physiological functions have extended from anti-obesity to variety of roles like formation of new blood cells and blood vessels, regulation of sympathetic tone and blood pressure maintenance, reproduction, bone mass regulation, enhancing insulin sensitivity and immunity[78]. (Figure:6)

FIGURE – 6

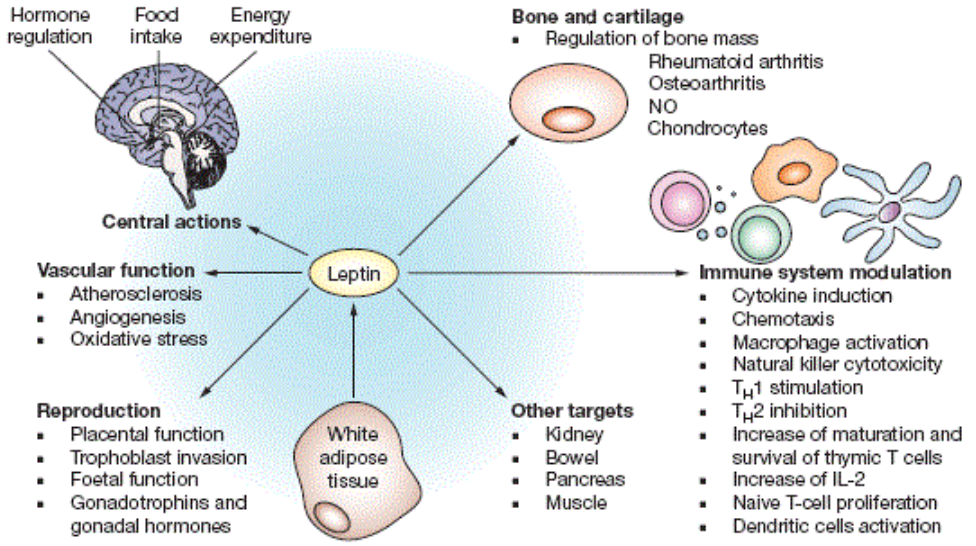
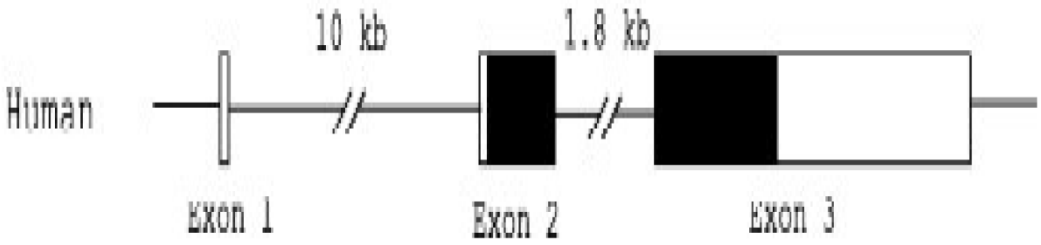


FIGURE – 7



Leptin Gene-LEP:

Leptin is a product of gene designated OB (Obese) or LEP(leptin). **Gene location:**

OB/LEP gene is located in Chromosome 6 in mice and Chr.7q.31.3 in humans[64].

Gene structure:

This gene has 3 exons separated by 2 introns in both rodents and humans[64]. The coding sequences of the gene is contained in exon 2 & 3 separated by an intron, approximately 2 kb. (Figure:7)

LEP encodes a 4.5 kilobase mRNA[63].

Gene expression:

The mRNA of the OB gene is highly expressed in adipocytes[79]. It has been found that the OB mRNA expression is not detected in human pre-adipocytes. It occurs following differentiation of 373L1 and 373F, 422A cells from fibroblasts to mature adipocytes [80,81]. The major factor determining the amount of ob mRNA expression is the size of the adipocyte. Larger the cell size more is its expression.

Leptin Receptors-LR:

They are the member of the cytokine receptor super family- class I. There are six forms of LR produced by variations in the splicing process[82-86].

The leptin receptor (LR) is coded by LEPR gene or ObR gene[86]. In mice this gene is present on chromosome 4 and in humans its location is on chromosome 1p [87]. It has also been designated as CD295 (cluster of differentiation 295).

LR Forms :

Till date six isoforms of LR have been discovered. They are Ob-Ra–Ob-Rf, or LepRa-LepRf. Out of them ObRb is the longest form. It has the complete intracellular signaling capability[86]. It is the only form that can exert its function through the Jak-Stat and MAPK pathways[88].

LR Structure:

LRa single-transmembrane-domain receptor of the cytokine receptor family. All the isoforms have an extracellular domain. This is the only portion of the receptors that is required for ligand binding[63,89,90] Structural analysis and functional studies of the receptor reveal that it prevails regularly as a dimer pair. A single leptin molecule binds to one LR in a reversible manner. Leptin OB-Rb receptor is found in hypothalamus. It is identical to that in

choroids plexus except that the intracellular domain has additional 269 amino acids. The extra cellular domain contains Trp-Ser-X-Ser-Trp motifs and the intracellular domain has docking sites for janus kinases (JAK), a family of tyrosine kinases involved in intracellular cytokine signaling and a signal transducer and activator of transcription (STAT) motif. OB-Rc and OB-Rd are speculated that they transport leptin through the BBB(Blood brain barrier). OB-Ra which is a soluble receptor functions as a transport protein contributing to binding and activation of circulating leptin[91,92].

Localization:

The LEPR gene is expressed significantly in the lung and adipocytes, moderately by kidneys , relatively less in heart, spleen, liver, brain and muscle[93]. Various tissues at the periphery express the short variant (LRa). ObRb- the long variant is expressed almost ubiquitously including the renal, cardiac and adrenal system [93] with maximum expression in hypothalamus.

Transport of leptin :

Leptin is considered to cross the BBB via the choroid plexus which has increased expression of LRa[94]. Thus this short variant is suggested to be one of the major transport route of leptin into the brain. This route is a single way transport system which gets saturated[95].

Mode of action:

It has been recognised that the action of leptin has more peripheral actions than thought earlier. Other than its endocrine effect linking the fat tissue with CNS, it also has autocrine and paracrine actions[24].

Secretion :

Leptin is predominantly secreted by fat cells into blood. Leptin exists in both free form (physiologically active) and complex form with binding proteins[96,97]. As the adiposity raises there is a relatively equal increase in circulating free leptin levels[96]. Its secretion is pulsatile[98] with diurnal-nocturnal variation, the highest levels observed during the night[98,99]. Its concentration is assayed by immuno-precipitation or radioimmunoassay[100-102].

FIGURE – 8

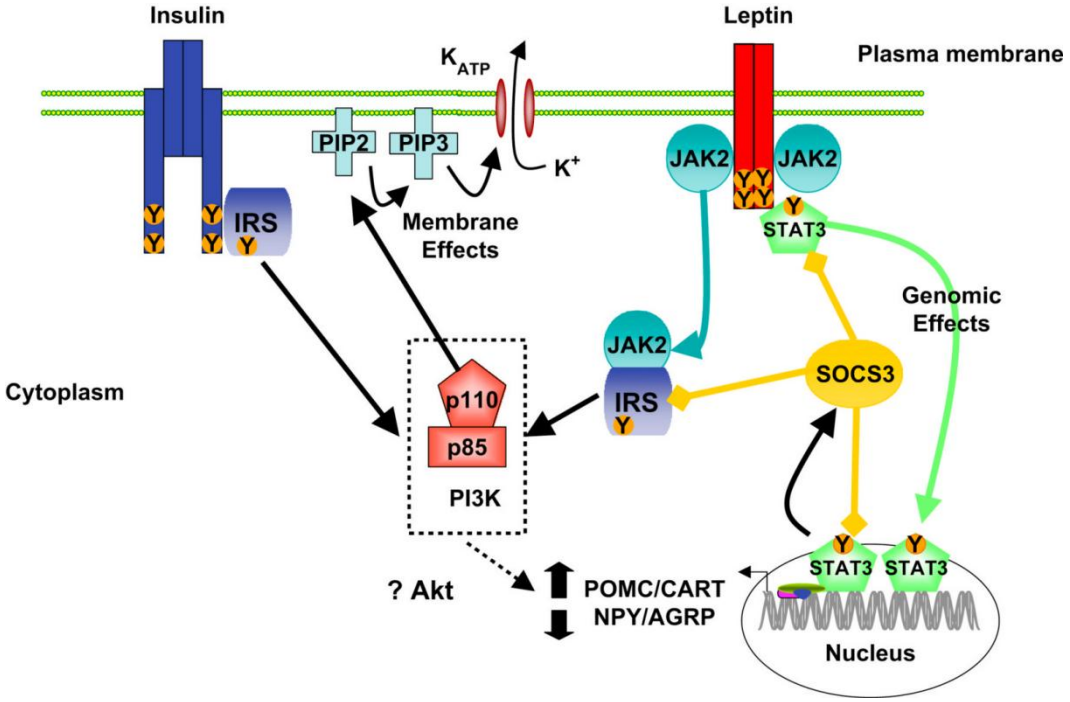
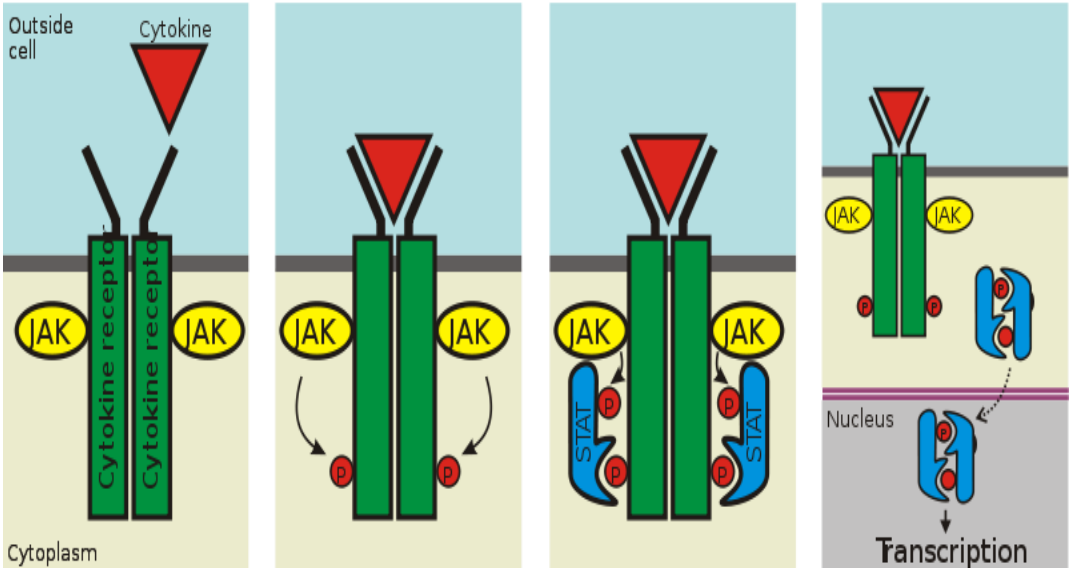


FIGURE - 9



Mechanism of action:

Most of the biological functions of leptin like appetite suppression, heat production and activation of the sympathetic nervous system are performed via stimulating the long isoform LRb[84,103]. This leads to activation of various intracellular signaling pathways like the JAK2/STAT3, MAPK, PI3K and AMPK pathways. (Figure:8)

On activation of LRb , one of the pathway JAK2 is stimulated (Janus kinase 2) [84,103]. This leads to phosphorylation of tyrosine residues present on the longer LR which in turn induces signal transduction and activator of transcription-3 (STAT-3) proteins[83,104-107]. Phosphorylated STAT-3 proteins dimerize. They then shift to the nucleus and promote transcription. (Figure:9) In addition, downstream of JAK, LR activation also stimulates phosphotidylinositol 3-kinase (PI3K) and Ras-mitogen activated protein kinase (MAPK) signaling pathways[3785,86,93,106-108]. Negative regulators of leptin signaling are SOCS-3 (suppressors of cytokine signaling protein) and PTP1b (protein tyrosine phosphatase 1b)[82-86].

In the CNS, activation of Jak2 via LRb leads to phosphorylation of 3 tyrosine moieties. This in turn results in 3 major signal routes:

1) Phosphorylation of Tyrosine-1138 attracts inactive STAT3 towards LRb-Jak2 complex, leading to addition of phosphate group and activation of

STAT3. Active STAT3 undergoes a shift into the nucleus leading on to transcription of genes.

2) Addition of phosphate group to IRS2 (insulin receptor substrate 2) results in stimulation of phosphatidylinositol 3-kinase (PI3K)

3) Addition of phosphate group to Tyrosine-985 attracts tyr phosphatase Shp2 leading to stimulation of MAPK pathway.

Stimulation of a variety of intracellular signaling routes via LR at various CNS areas suggest that there is a divergent regulation of appetite, energy expenditure ,BP in obesity.

Physiological roles of leptin :

Leptin seems to play a variety of functions ranging from regulation of energy utilization, as a signal of metabolic status, growth inducer in different types of tissues, permissive factor for puberty, regulation of metabolism during pregnancy, interaction with various hormones and modulators of energy like insulin, glucocorticoids, glucagon, growth hormone,IGFs.

Satiety and energy balance:

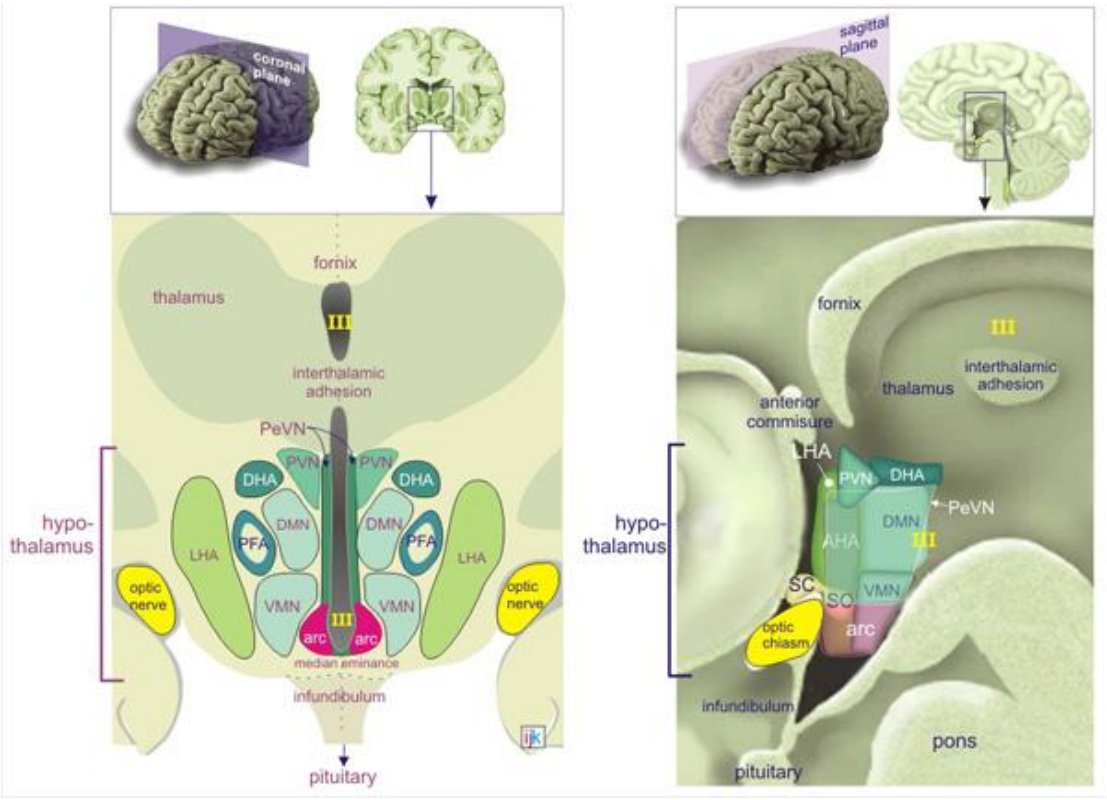
Leptin is an indicator of energy balance and provides satiety signal. On the contrary, according to some authors foremost function of leptin is to serve as

the “starvation indicator” to maintain sufficient lipid reserves for withstanding the states of deficient energy rather than being a “satiety indicator” to control excess fat deposition when there is lot of energy[109,110.]

In brain leptin acts on its receptors in the hypothalamus and either activates or inhibits various pathways.

Leptin enhances the production of α-MSH, which causes suppression of appetite. This leads to control on appetite on a long-term manner while the other appetite suppressants like cholecystokinin (CCK) has rapid inhibition and PYY3-36 has slower suppression of appetite. Leptin counteracts the feeding stimulants like neuropeptide Y and anandamide. Thus the absence or defect of leptin or its receptor results in excessive appetite and finally to increased adiposity. Various research works have revealed that leptin is more sensitive to starvation than to overfeeding[111]. Its level gets decreased when on a fast or on a very-low-calorie diet (VLCD)[112-114]. Thus by regulating appetite leptin controls the energy balance[115,116]. Melatonin regulates leptin levels during night. Role of melatonin is controversial. One study suggests a raised level of melatonin will lead on to decreased concentrations of leptin[117]. Another study reveals that there is a decrease in appetite during sleep as melatonin enhances leptin concentrations[118].

FIGURE – 10



Mechanism of action of leptin in CNS:

LRs are expressed in various hypothalamic nuclei like the arcuate nucleus,PVN, VMH AND DMH[119]. (Figure:10)

Arcuate nucleus is thought to be the prime target for the action of circulating leptin into a neuronal response[120]. On injecting leptin focally on this site it suppresses intake of food. After destruction of the arcuate nucleus, if leptin is administered directly into brain there is no effect on appetite or

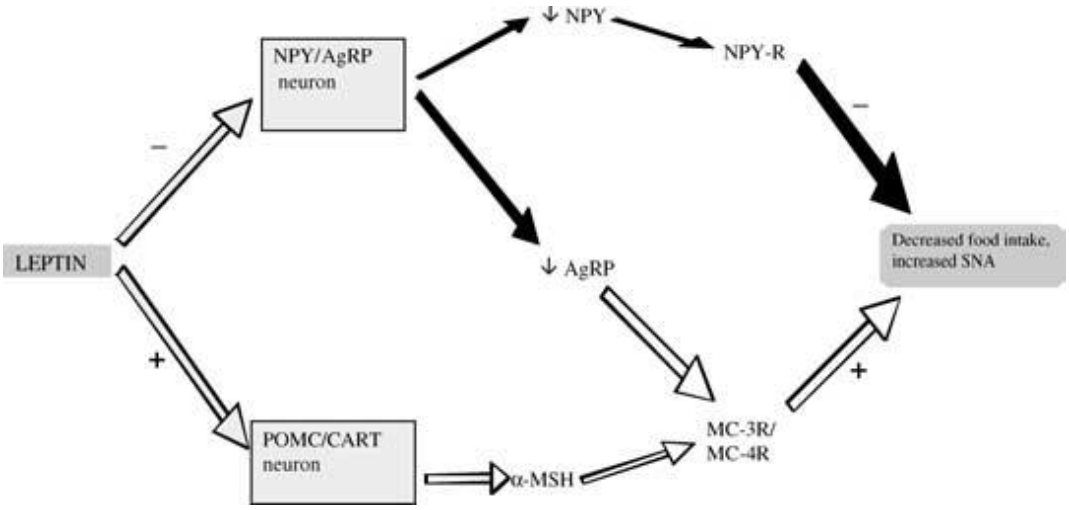


FIGURE 11. interaction of leptin with Vneuropeptide y (npy)/agouti-related protein (agrp)- and proopiomelanocortin (pomc)/cocaineand amphetamine-regulated transcript (cart)-containing neurones in the hypothalamic arcuate nucleus leptin stimulates the pomc/cart catabolic pathway and inhibits the npy/agrp anabolic pathway, leading to an increase in sympathetic nerve activity (sna) and reduced food intake. *α*-msh, *α*-melanocytestimulating hormone; mc-3r, melanocortin receptor 3; mc-4r, melanocortin receptor 4; npy-r, neuropeptide y receptor.

sympathetic nerve activity [121]. Signals from arcuate nucleus are relayed onto PVN and lateral hypothalamus[119].

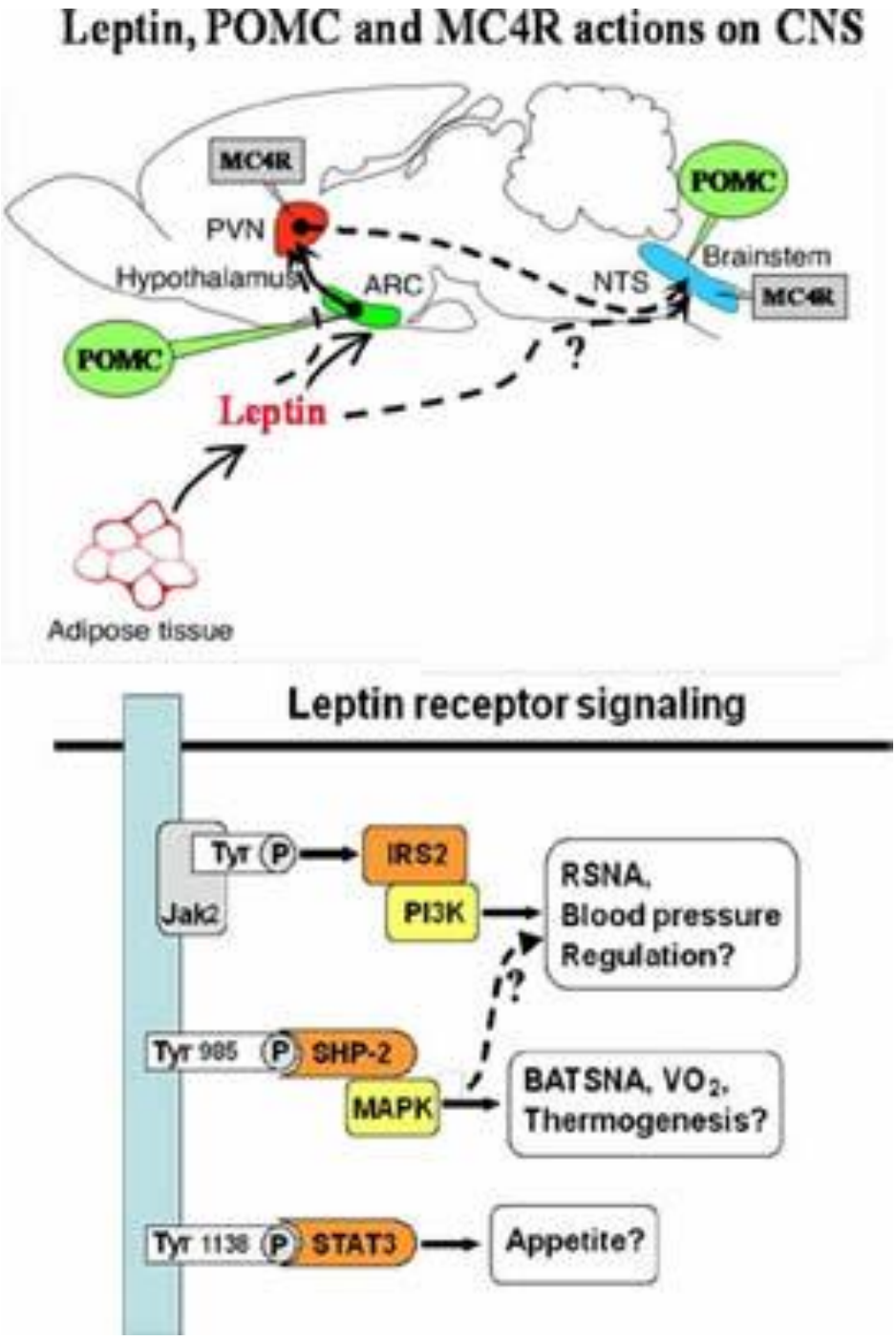
Leptin involves different neuronal circuits for its anorexigenic, metabolic and sympathetic actions. It triggers the catabolic pathway proopiomelanocortin (POMC)/cocaine-and amphetamine-regulated transcript (CART) neurons and inhibits the anabolic pathway represented by the neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons. Both these neurons relay onto PVN and lateral hypothalamic area[122]. POMC/CART neurons also project to the adrenergic preganglionic neurons present in the medulla and spinal cord[122]. (Figure:11) . The various pathways involved in mediating leptin's action are as follows:

1. Melanocortin system:

One of the pathways for leptin's action on CNS is the melanocortin system [119]. Leptin acts over LRs on the POMC neurons present in arcuate nucleus to produce melanocortins (eg. alpha-melanocyte stimulating hormone or α-MSH) . (Figure:12) This in turn act on a variety of melanocortin receptors. There are 5 such receptors. MC-1R, MC-2R, MC-3R, MC-4R, MC-5R MC-3 and 4R are the predominant forms in CNS.

MC-4R is important in regulating the energy status and any destruction to its gene causes increased appetite and excess weight gain in mice[123]. Also when hypothalamic MC-4R receptors are stimulated they increase the SNS activity to

FIGURE - 12


Leptin, POMC and MC4R actions on CNS
MC4R
PVN
Hypothalamus
ARC
POMC
Leptin
Adipose tissue
POMC
Brainstem
NTS
MC4R
?
Leptin receptor signaling
Tyr
P
Jak2
IRS2
PI3K
RSNA,
Blood pressure
Regulation?
?
Tyr 985
P
SHP-2
MAPK
BATSNA, VO2,
Thermogenesis?
Tyr 1138
P
STAT3
Appetite?

BAT and kidney[124]. Inhibition of MC-4R inhibits the sympathoexcitatory properties of leptin over the renal system while BAT remains unaffected[124]. This selective inhibition suggests that control on sympathetic nerve activity by leptin is in a tissue specific way via multiple signalling routes.

2. Neuropeptide Y :

NPY which is produced from neurons of the arcuate nucleus acts on PVN and lateral hypothalamus. NPY increases food intake and enhances obesity[125]. These effects result from action on NPY-Y1 & NPY-Y5 receptors. Also the NPY neurons produce a potent blocker of the melanocortin receptors 3&4 named agouti related peptide (AgRP). This AgRP gets increased during fasting and during deficient leptin levels. This augments food intake and reduces the energy expense by antagonizing melanocortin receptors [126]. Leptin blocks NPY gene transcription and translation and thereby reduces obesity[127]. Administration of neuropeptide Y to animals via intracerebroventricular route decreases sympathetic activity to interscapular brown adipose tissue and kidney[128]. Thus, blockage of neuropeptide Y signalling route seems to be one of the key mechanisms of central action of leptin to regulate energy status.

3. Corticotrophin Releasing Factor(CRF):

In brown adipose tissue (BAT) the sympathetic activation by leptin seems to be carried out by CRF for the reason that this activation is blocked by an

antagonist to CRF receptor [129]. This adds to the possibility that leptin controls SNS at different sites in a tissue specific way through a variety of signalling pathways.

Sexual dimorphism:

In human-beings, leptin displays a difference in their concentrations in both sexes . This sexual dimorphism is evident in both ob mRNA expression[130] and in the correlation between concentrations of leptin and body fat mass[131,132]. Leptin concentrations are greater in women when compared to men[131,133]. Though with similar BMI, females tend to have greater adipose tissue mass than males, this fact doesn't contribute to the sexual dimorphism of leptin levels. It seems to arise independent of adiposity[131,133].

A couple of reasons could be put-forth for this dimorphism.

1. One reason could be that, females have a greater subcutaneous to omental fat ratio [134]. This higher ratio is significantly associated with leptin expression in women[130]. Thus reflecting that, these gender variations are due to the local distribution of fat mass and local leptin expression.
2. Another reason could be due to influence of sex hormones over leptin [131,133]. It is proposed that such gender dimorphism in the production of leptin are due to the stimulatory effects of estrogen or due to inhibitory

effects of androgens[80 135-137], while such an association among the above hormones was inconsistent in another study[138].

Leptin and BMI:

Leptin levels and its expression increases as the amount of triglyceride stores in adipose tissue increases[100,139]. Serum leptin correlates specifically with the body mass index and percentage of fat in humans[96,97]. Recent studies show that inspite of no changes in the adiposity, there is a peak rise in serum leptin levels due to increased caloric intake [140,141] but there is no increase acutely in the postprandial state[99]. Whereas leptin expression and its concentration decreases intensely during starvation[142,143]. Therefore, alterations in adipose tissue mass and sudden alterations in energy homeostasis seem to contribute to regulation of concentrations of leptin. It is found that the adipocyte differentiation is in turn controlled by CCAAT/enhancer-binding protein a (C/EBPa)[144,145]. This protein is a transcription factor which positively regulates the promoter region of LEP.

Hormonal influence on leptin levels:

Insulin increases the production of leptin and its secretion from human adipocytes [146,147]. Following a high calorie intake, there is increase in insulin

FIGURE - 13

Factors That Regulate Circulating Leptin Levels
Factors promoting leptin secretion
Excess energy stored as fat (obesity)*
Overfeeding*
Glucose
Insulin
Glucocorticoids
Estrogens†
Inflammatory cytokines, including tumor necrosis factor-α and interleukin-6 (acute effect)
Factors inhibiting leptin secretion
Low energy states with decreased fat stores (leanness)*
Fasting*
Catecholamines and adrenergic agonists
Thyroid hormones
Androgens†
Peroxisome proliferator–activated receptor-γ agonists‡
Inflammatory cytokines, including tumor necrosis factor-α (prolonged effect)

* Denotes major factor influencing leptin levels.
† Women have higher levels than men, even after adjustment for body mass index and the effects of sex steroids, mainly because of different body-fat distribution (9, 10).
‡ Unlike in animals, peroxisome proliferator–activated receptor-γ agonists decrease leptin gene expression but increase subcutaneous fat mass in humans; thus, the net effect is null.

which stimulates rise in leptin levels in a dose-dependent manner[148]. This is potentiated by high cortisol levels[146].

Hyperinsulinaemia over a long term, increases leptin production and the serum leptin concentrations in humans but however this effect is not seen in acute rise in insulin levels [149-151]. Serum leptin levels were increased in hyperinsulinaemic lean individuals with insulin resistance[152] while subjects with NIDDM and PCOS, insulin resistant conditions did not have an increased leptin levels[153,154]. However, it is unclear whether this association with higher serum leptin levels is independent of obesity.

High levels of glucocorticoids increase leptin expression[155]. Higher serum leptin levels are seen in Cushing's syndrome[156] independent of body adiposity. Positive regulators of leptin are mainly cytokines which include TNF[154] and interleukin1 and the negative regulators are beta3-adrenergic agonists and cyclic AMP (cAMP)[157]. Decreased serum leptin levels are associated with cigarette smoking possibly due to adrenergic activation [158]. However, hyperthyroidism another hyperadrenergic state did not have any effect on leptin levels[159,160]. (Figure:13)

Selective leptin resistance :

Serum leptin levels are significantly raised in obese persons when compared to thin subjects[100]. This seems to be due to the resistance to metabolic

FIGURE – 14

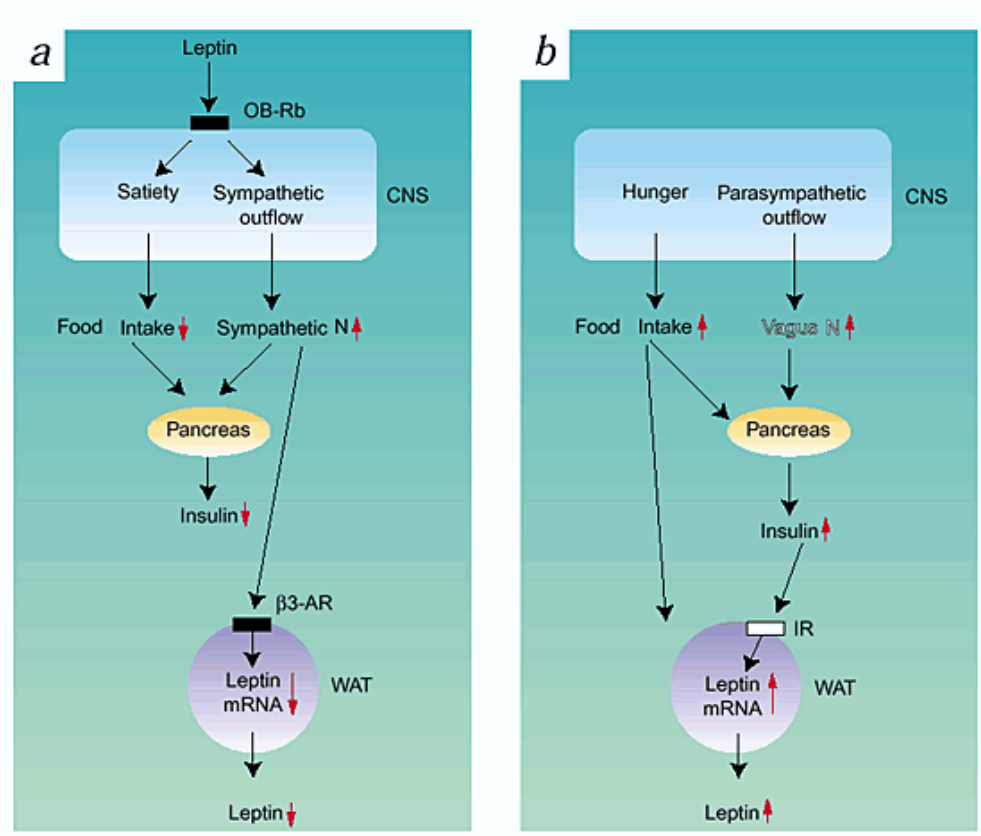
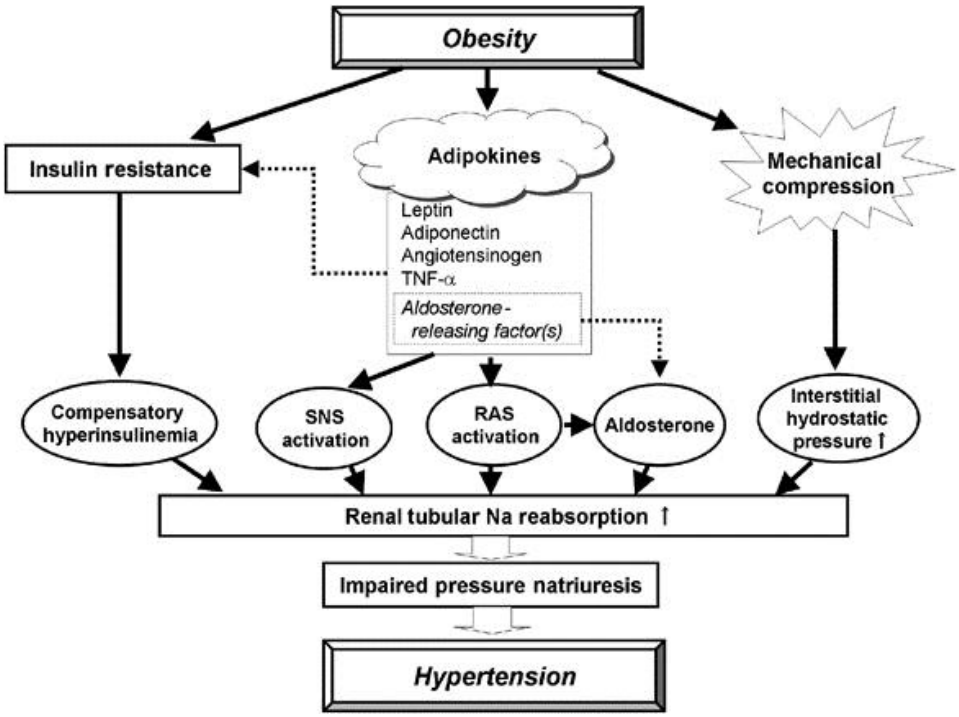


FIGURE – 15



effects of leptin as hyperleptinemia cannot regulate the fat mass in obese persons. In animal studies it has been demonstrated that in obesity, there is resistance to the suppression of appetite by leptin but not to its action on SNS [161,162]. Also the neutral action of leptin in maintaining the normal vascular tone is impaired in chronic hyperleptinemia found in obesity. Increased leptin levels may enhance the smooth muscle cell proliferation in the blood vessels[163] . This concept was termed as "selective leptin resistance".

Leptin and insulin:

Leptin has been found to enhance the sensitivity of insulin & simultaneously lowering its production. Sequentially, insulin enhances secretion of leptin, thereby setting up an 'adipo-insular axis'. (Figure:14)

Leptin increases insulin sensitivity at its physiological levels. However in case of hyperleptinemia as in leptin resistance it is shown to cause insulin resistance. (Figure:15)

Insulin resistance and hyperinsulinemia per se are linked to hypertension[164-166]. This is due elevated adrenergic activity[136,137] and renal sodium reabsorption. Thus the most probable cause for the link between obesity and hypertension is insulin resistance and the consequent hyperinsulinemia[167]. Elevated leptin levels also seem to be responsible for increased blood pressure

FIGURE – 16

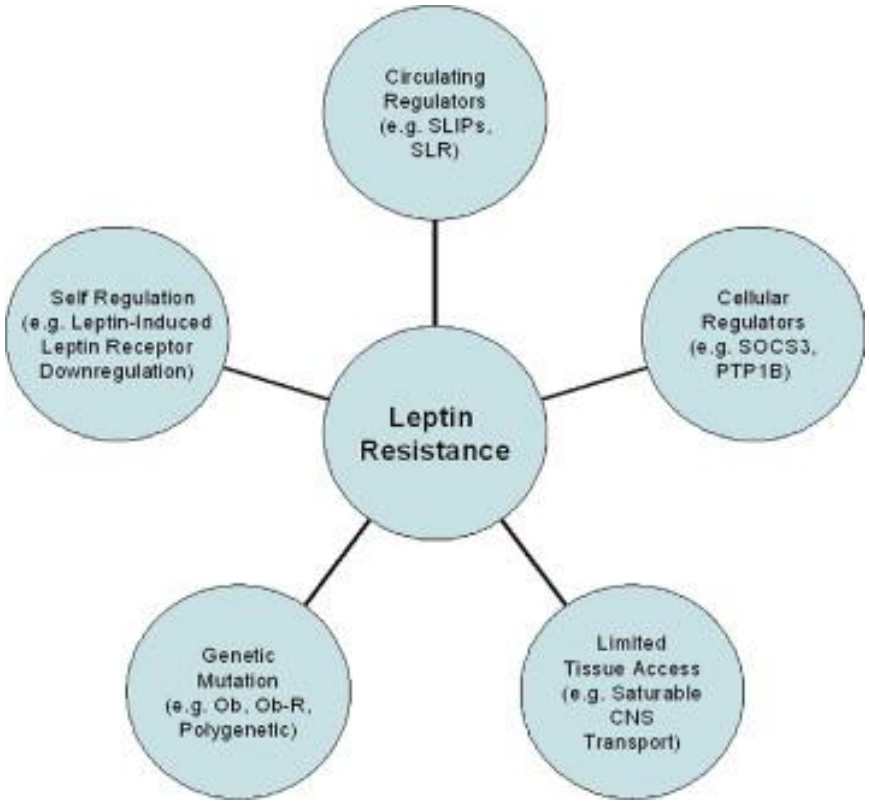
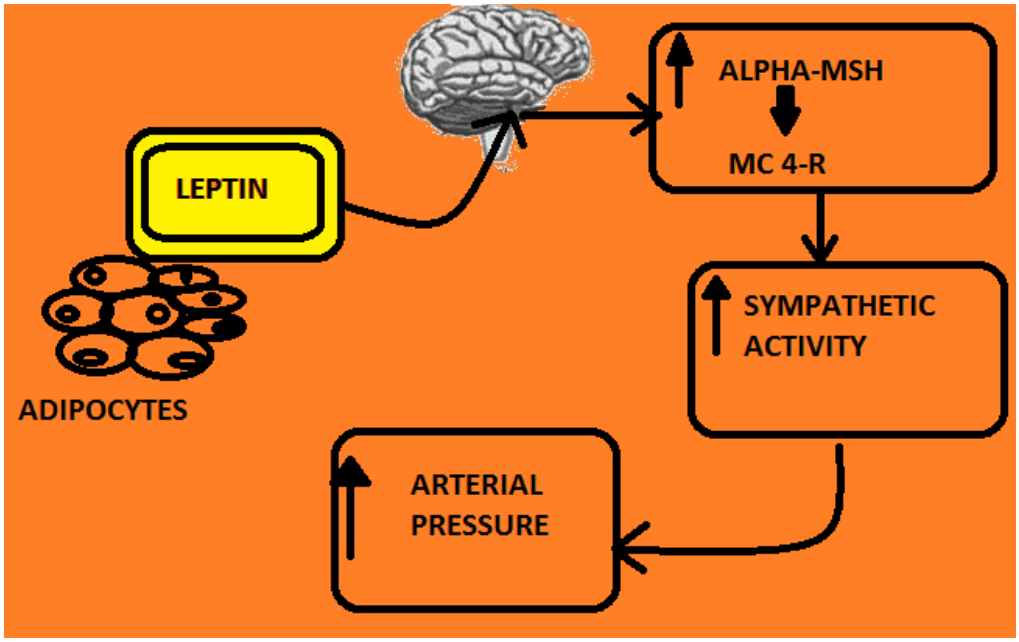


FIGURE – 17



by adrenergic[168,169] or renal[170] mechanisms and has been highly suggested as another possible link for hypertension.

Leptin Resistance:

Possible factors involving leptin resistance[84,171-174] (Figure:16)

1. Saturation of the leptin transport carriers into the central nervous system.

2. Changes in leptin signalling routes within the hypothalamus eg. phosphorylation of serine residues in IRS-1(insulin receptor substrate-1).

3. Activation of inhibitors to leptin signaling pathways like SOCS3.

4. Defects in the leptin receptor but their mutations are rare in humans.

Differential actions of leptin:

It is suggested that there is differential regulation of energy status and SNS activity[175].

Leptin has differential actions over sympathetic system controlling the metabolic and cardiovascular functons. This is revealed by the finding that the

stimulation of baroreceptors specifically blocks renal sympathoactivation by leptin but doesnot alter its sympathoactivation in the BAT [176]. It is a found that leptin's sympathoactivation in the kidneys play a major role in its pressor effects and is regulated by the baroreflex. Leptin's sympathoexcitation in the brown adipose tissue contributes to its metabolic regulation. These two effects of leptin involve different neuronal pathways.

Melanocortin- 4 receptor antagonists inhibit renal sympathoactivation by leptin but doesn't affect leptin's BAT sympathoactivation[124]. BAT sympathoexcitation by leptin is blocked by a CRF receptor antagonist[129]. The varying predilection to hypertension in obese subjects could be due to differential resistance to leptin's action in controlling metabolic status and SNS activation. Thus this could be the possible reason for elevated leptin levels in obese hypertensives to be much more greater than those with obesity,normal blood pressure[177]. Serum leptin concentration and bp were related in graded positive manner independent of BMI, abdominal adiposity and insulin resistance[178].

Research studies on humans suggest that there is a significant relation between increased leptin levels and increased bp in both sexes even after adjusting for the influence of body mass index and insulin resistance [179,180]. Higher plasma leptin levels have a positive association with increased bp after controlling the influence of age, sex, race/ethnicity,BMI,smoking,DM and

cholesterol levels[181]. There is possibility of strong link between leptin and the RAS over the maintenance of blood pressure. This interaction was postulated by recent studies where there was a fall in leptin concentrations by administering ACE inhibitors and AT receptor blockers[182,183]. Melanocortin system may also play a potential part in leptin's maintenance of blood pressure[124]. It is suggested that the MC4R might be involved in the SNS activity of leptin[184]. (Figure:17) Mutation in MC4R gene showed poor association of raised bp in obesity[185].

Leptin and heart:

Leptin might be involved in cardiovascular functions. Chronic hyperleptinemia may indirectly lead to cardiac disorders through sympathetic overactivation, pressor effects, increased platelet aggregation, impaired fibrinolysis and proangiogenic actions[75,174,186,187] and inflammatory response through C-reactive protein stimulation(CRP)[188,189].

Leptin and blood vessels:

Leptin promotes angiogenesis by increasing vascular endothelial growth factor (VEGF) levels.

Role of leptin as an Inflammatory marker

The leptin has a significant function in imparting inflammatory response. Other inflammatory inducers like sleepdisorders, stress, caloric limitation and

adiposity seem to acutely affect leptin levels also [190-192]. Leptin specifically responds to adipose-derived inflammatory cytokines. Leptin resembles IL-6 both by structure and function[66,191,193]. Plasma leptin affects the HPA axis, indicating its part in stress response[194]. Increased leptin levels correlate with increased WBC counts in both men and women[195]. Unlike IL-6, leptin is not affected by exercise. Leptin stimulates angiogenesis, inflammation and mitogenesis[196].

Role of leptin in sodium handling:

Leptin seems to be a natriuretic hormone. It helps in excretion of sodium and water by functioning in the renal tubules. In chronic hyperleptinemia there is a predilection to an enhanced SNS activity which leads to an additional rise in bp and a blunted natriuretic effect with reduced clearance of sodium and water. The reasons for this blunted natriuretic effect are not fully identified. Suggested mechanisms are the down regulation of LR [75,197] ,post-receptor signalling alterations[75,84,106], increased breakdown of NO by oxidative stress[198] ,or over stimulation of the efferent RSNA causing antinatriuresis[199]. An important physiological role of leptin is to control salt and water balance. Also dietary sodium has a positive correlation with synthesis and secretion of leptin [200]. It is found that under varied states of physiology, the response to leptin's natriuretic effect and vascular tone changes.

FIGURE – 18

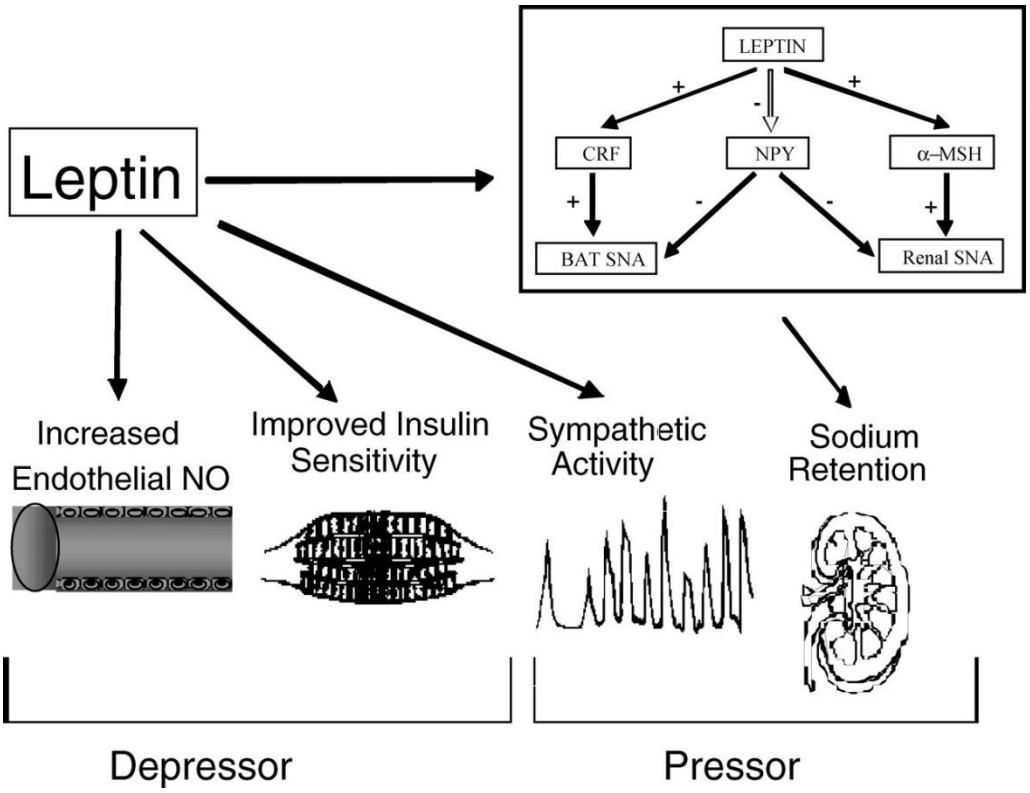
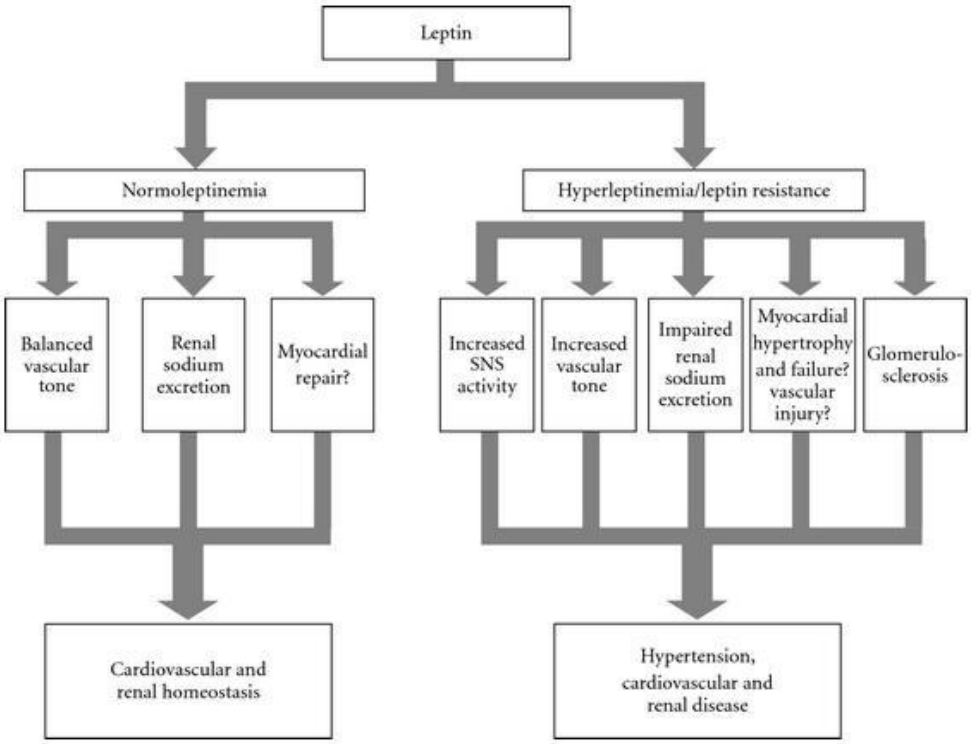

LEPTIN
+
-
+
CRF
NPY
α–MSH
+
-
-
+
BAT SNA
Renal SNA
Leptin
Increased Endothelial NO
Improved Insulin Sensitivity
Sympathetic Activity
Sodium Retention
Depressor
Pressor

FIGURE – 19


Leptin
Normoleptinemia
Hyperleptinemia/leptin resistance
Balanced vascular tone
Renal sodium excretion
Myocardial repair?
Increased SNS activity
Increased vascular tone
Impaired renal sodium excretion
Myocardial hypertrophy and failure? vascular injury?
Glomerulo-sclerosis
Cardiovascular and renal homeostasis
Hypertension, cardiovascular and renal disease

Role of leptin in blood pressure regulation:

Physiologically leptin is known to have both pressor and depressor effects. (Figure:18) The depressor effects are believed to be due to natriuretic effect of leptin, stimulation of endothelial nitric oxide which has vasodilatory effect and indirectly by increasing the insulin sensitivity. The pressor effects are due to increased SNS activity and due to adverse shifting of pressure-natriuresis curve[201,202]. Although leptin has dual and opposite roles over blood pressure regulation, in order to maintain a normal vascular tone usually the pressor effects predominate the depressor effects in vivo. Thus when there increased levels of leptin as in leptin resistance, it results in increased activation of the renal sympathetic system coupled with decreased natriuresis[203]. (Figure:19) Thus hyperleptinemia is likely to produce hypertension[204].

Leptin activates the SNS directly either by peripheral or by central actions on hypothalamus[205] . According to study reports on animal models, leptin is believed to activate the sympathetic nervous system to produce vaso-active response[205]. On the contrary, there were no changes in blood pressure when leptin was injected on a short term basis. This postulates the probability of simultaneous stimulation of counter-regulatory mechanisms like NO-dependent vasorelaxation, EDHF and natriuresis which are known to cause vasodilation [206,207]. This vasodilatory action is impaired in obese animals[208]. In such obese animals leptin levels were highly elevated. This inturn leads to increase in blood

pressure by increased sympathetic nervous activity, sodium retention, nitric oxide deficit, increase Na-K-ATPase in kidneys, and excess synthesis of endothelin which is a potent vasoconstrictor[208-210]. Also an increase in activation of SNS and thereby blood pressure are also brought about by an increase in CRF in brain[129].

In accordance to the above factors, leptin deficiency in homozygous individuals were associated with obesity and also defective SNS activity leading to decreased blood pressure [211].

Mechanism involving hypertension:

Chronic increase in leptin can raise BP. This increase in BP is completely destroyed by α-&β-adrenergic inhibitors. Thus it is highly suggestive that leptin mediated rise in BP is by SNS activation[212,213]. Increase in leptin levels increases BP in a slow fashion stating that the rise of SNS activity is not sufficient to produce a striking vasoconstriction. Instead, the enhanced SNS activity amplifies BP through the deliberate renal mechanism. The effects of leptin on increased BP are greatly exacerbated only when accompanied by impaired NO synthesis[212,213].

Role of POMC in hypertension:

CNS POMC-MC3/4R system not only regulates energy balance but also cause SNS activation and hypertension. Long term activation of this system causes these effects whereas its inhibition lowers BP.

MC4R is predominant in PVN and NTS [214]. It is a G-protein-coupled receptor which acts by increasing activity of adenylate cyclase[215]. Individuals with deficiency of this receptor are less prone to hypertension inspite of marked obesity[185] .

POMC neurons are located in immediate contact with hypothalamus along with LR[215]. Leptin augments the production of POMC which on activation leads to synthesis of α-melanocyte-stimulating hormone (α-MSH)[215].

In an animal study, deletion of LR specifically in POMC neurons and then chronic infusions of leptin didn't result in an increased bp but there was no effect on appetite. This highly suggests that the role of leptin in regulating blood pressure is through the stimulation of POMC-MC3/4R route [216].Also stimulation of RSNA[124] and thereby increasing BP[217] by leptin were completely abolished by pharmacological antagonism of MC3/4Rs.

These effects on BP are carried out predominantly by MC4R [218]. However inhibition of MC4R did not lower the SNS activity of leptin in BAT indicating a different neuronal pathway involvement other than that involving the RSNA and BP[124].

Leptin gene and hypertension:

From animal studies it has been observed that mutations of LEP(OB) leads to profound obesity. In humans, such mutations are extremely uncommon in LEP gene. However many studies show a significant association between single nucleotide polymorphisms, tandem repeat polymorphisms of leptin gene and hypertension. Duanduan Ma et al. observed six LEP SNPs to be significantly associated with hypertension. A tetranucleotide (TTTC)n repeat polymorphism was identified in the 3'UTR of leptin gene(LEP-tet) by Shintani et al. Polymorphism with fewer tetranucleotide repeats (<160 bp) were found to be significantly associated with hypertension[219]. This association was not dependent on BMI and insulin resistance. Similar results were obtained in few other studies [220,221]. Another similar study was carried out in India which also revealed a strong association between this tetranucleotide repeat polymorphism and EH but was not independent of obesity[222]. Few other studies were in contrast to these results showing nil association between LEP-tet polymorphism and hypertension[223,224]. One another study on linkage analysis conducted in African-Americans between LEP and HT showed no significance[225].

In another study, LEP-tet polymorphism was also associated with pulse pressure and carotid intimal thickness[226]. Also few authors studied this polymorphism,to find out any association with diabetes[227] and obstructive sleep apnea[228] and found no association.

Aim of the Study

AIM OF THE STUDY

On reviewing the physiological role of leptin and its association with essential hypertension and the possible association of leptin gene tetra-nucleotide repeat polymorphism at the 3'UTR with essential hypertension , the study has been taken up with the keen interest to establish the following aims and objectives:

1. To know the distribution of genotypes & their frequencies among hypertensives and apparently healthy controls.
2. To know the frequencies of the alleles(Class I &Class II) among hypertensives and apparently healthy controls.
3. To find an association between class I allele and its associated risk of essential hypertension.
4. To estimate serum leptin levels in subjects with essential hypertension and apparently healthy controls.
5. To know the relation between genotypes and serum leptin levels among hypertensives and apparently healthy controls.
6. To know the relation between the genotypes and BMI among hypertensives and apparently healthy controls.
7. To determine whether there is any significant association between the genotypes and essential hypertension independent of obesity.

8. To determine if there is any correlation between serum leptin levels and lipid parameters.
9. To estimate fasting serum insulin levels in subjects with essential hypertension and apparently healthy controls.
10. To calculate HOMA-IR among hypertensives and apparently healthy controls and to assess any link between the genotypes, leptin levels and insulin resistance.

Materials and Methods

MATERIALS AND METHODS

A case-control study was carried out between April'11 – September'12. It was done in 2 groups, namely, cases with essential hypertension and controls who were apparently healthy.

Cases group:

This group comprised of 100 subjects with essential hypertension who were attending the hypertensive clinic and the master health checkup-OPD at Rajiv Gandhi Government General Hospital, Chennai.

Based on the following inclusion and exclusion criterias subjects of this group were selected.

Inclusion criteria:

Patients with confirmed diagnosis of essential hypertension based on history and blood pressure measurement using sphygmomanometer.

Exclusion criteria:

- Patients of essential hypertension with associated diabetes mellitus
- Clinical information or lab data indicative of secondary hypertension

- Valvular heart disease, arrhythmias, renal disease, coronary artery disease, cerebrovascular disease,endocrine disorders

- Significant medical disorders:

    a. Cardiovascular diseases
    b. Thyroid disorders
    c. Renal diseases
    d. Adrenal disorders
    e. Hepatic diseases
    f. Neurological disorders
    g. GastroIntestinal disorders
    h. Recent infection, inflammation(past 1 month)
    i. Recent drug intake
        * Lipid lowering drugs,
        * Non-Steroidal Anti Inflammatory Drugs,
        * Hormone Replacement Therapy,
        * Immunosuppressives,
        * Cox -2 inhibitors,
        * Oral Contraceptive Pills,
        * Mineralocorticoids,
        * Bromocriptine,
        * MonoAmineOxidase inhibitors

Control group:

The group comprised of 100 apparently healthy subjects with no significant medical illness. They were selected from the patients attending the master health checkup-OPD at Rajiv Gandhi Government General Hospital, Chennai.

Sample collection:

5 ml of peripheral venous blood was collected after an overnight fasting of 8-12hrs from all the study subjects. Blood was withdrawn under sterile conditions with disposable syringes from the cubital vein of the subjects. 2 ml of the blood was transferred to a potassium EDTA tubes and the remaining 3ml of blood into plain tubes. Plasma and serum were separated from the above tubes respectively.

Serum separated from the plain tubes was centrifuged at 2500 revolutions per minute for 5 minutes, to get clear serum without any cells. 1mL of serum was transferred into 1.5mL eppendorf. This was stored at -20°C for measuring the concentrations of leptin and insulin. The remaining serum was used to measure urea and creatinine within 6 hours of blood collection by enzymatic methods using commercial kits.

EDTA tube was centrifuged at 2000 rpm for twenty minutes to get the buffy coat for DNA extraction. The plasma samples were analysed for glucose estimation and lipid profile estimation on the same day within an hour of collection.

DNA extraction was done on the same day and extracted DNA was stored at -20°C.

Height (in cms) and Weight (in kgs) of the subjects were measured to calculate the body mass index.

BUFFY COAT SEPARATION

Buffy coat was separated by centrifugation of EDTA tubes at 2000 revolutions for 20 minutes. Buffy coat was transferred to 2mL eppendorf and was used for DNA extraction. Plasma separated was used for glucose and lipid profile estimation.

BIOCHEMICAL MARKERS

Plasma glucose, serum urea, creatinine, total cholesterol (TC), triglyceride(TGL) high density lipoprotein cholesterol (HDL-c)concentrations were estimated enzymatically using kits & Chem-7 semi-autoanalyser at Centralized Biochemistry Laboratory at Rajiv Gandhi Government General

Hospital, Chennai-3.Low density lipoprotein cholesterol (LDL-c) was calculated using Friedwald's formula.

DNA EXTRACTION BY MODIFIED HIGH SALT METHOD[229]

RBC Lysis:

- 400µL of buffy coat in a 2mL eppendorf is mixed with 1.6mL of 0.17M ammonium chloride and mixed by inversion until red cells are lysed for about 10 minutes

- The cells are centrifuged at 4000rpm for 10minutes.

- The white cell pellet is washed with 800µL of 0.17M ammonium chloride solution. The procedure is repeated till a clear white cell pellet is obtained.

WBC Lysis

- To the pellet 500 µL of TKM I solution is added. It is centrifuged at 10,000rpm for 10minutes.

Nuclear Lysis

- Discard the supernatant. To the pellet add 500 µL of TKM II solution. To that add 300 µL of 6M Nacl and 50 µL of 10% SDS.

- Mix well (vortex), Centrifuge at 10,000 rpm for 10 minutes.

- Save the supernatant. Transfer it to 1.5mL eppendorf.

DNA Precipitation

- To the supernatant double the volume of 100% ethanol is added.

- The sample is stored at -20◦C for 1 hour.

- Then it is centrifuged at 10,000 rpm for 20minutes at 4◦C in a refrigerated centrifuge.

- The supernatant is discarded. To this 500 µL of 70% ethanol is added. The pellet is mixed and centrifugation done at 10,000 rpm for 10minutes at 4°C.

- Remaining supernatant is discarded & the pellet is air dried.

Storage

- To the pellet 30 µL of LTE buffer is added and the extracted DNA is stored at -20◦C for future use.

Identification

- Extracted DNA was identified by 0.8% agarose gel electrophoresis with a constant voltage of 7V/ cm and comparison with a known molecular weight 1kb DNA ladder. (Figure:20)

Concentration of extracted DNA:

- Concentration of extracted DNA was estimated using UV spectrophotometer at 260 nm. The absorbance at 260nm was 0.0204.
- Concentration was calculated using the formula :

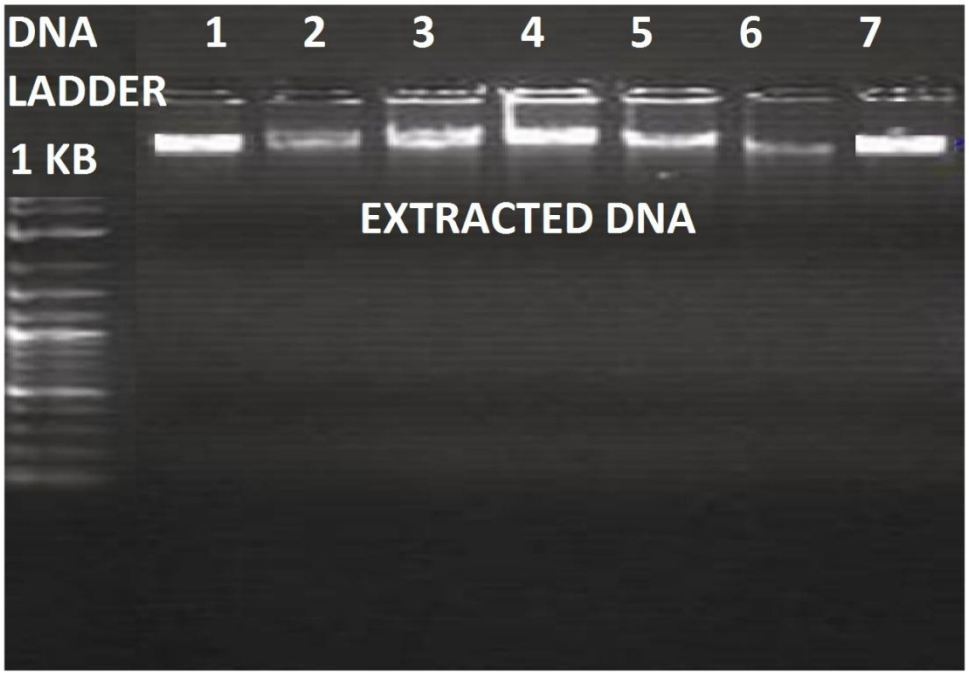
  1 OD is equivalent to 50 µg/mL.

  Conc. of DNA = absorbance X 50 µg/mL X dilution factor

  = 0.0204 X 50 X 100

  = 102 ng/µL
- Purity of extracted DNA was assessed by 260nm/280nm ratio and it was found to be >1.7.

FIGURE – 20



POLYMERASE CHAIN REACTION

A tetra-nucleotide repeat(TTTC)n polymorphism in the 3'-UTR of LEP gene was identified by polymerase chain reaction.

Primer sequences were human OB forward and human OB reverse

- Forward primer – 5'-AGTTCAAATAGAGGTCCAAATCA-3'
- Reverse primer- 5'-TTCTGAGGTTGTGTCACTGGCA-3'

Reconstitution of the Primers:

Primers are supplied in lyophilized form. Autoclaved distilled water is used to prepare 100 × concentrations i.e. 10times the molecular weight of

primer is the volume of water required to prepare 100 × concentrations which is 100µmolar solution.

- From this stock solution 10 × concentration is prepared as the working solution for PCR.

MASTER MIX:

- Genei Red Dye master mix in the following composition was used.
- Master Mix consists of a unique inert red dye in addition to basic components necessary for PCR.

* Reaction buffer consist of Tris Hydrochloric acid- 10 mM at pH8.3

  Potassium chloride - 50 mM

* MgCl - 1.5 mM acts as catalyst.

* dNTP's were used in a concentration of 2.5mM each.

* Taq polymerase in a concentration of 1.5 U.

* Primers were used in a concentration of 5 pmol and DNA was used in a concentration of 200ng.

* PCR was carried out in a reaction volume of 25 µL with the following components;

PCR master mix – 12.5 µL

Forward primer – 0.8 µL

Reverse primer – 0.8 µL

DNA – 2 .0µL

Distilled water – 8.9 µL

Total – 25 µL

* Amplification was carried out in an Mc Genei thermal cycler with the following cycling conditions.

Initial denaturation – 94^0 C -3min

35 cycles of

Denaturation – 94^0C – 30sec

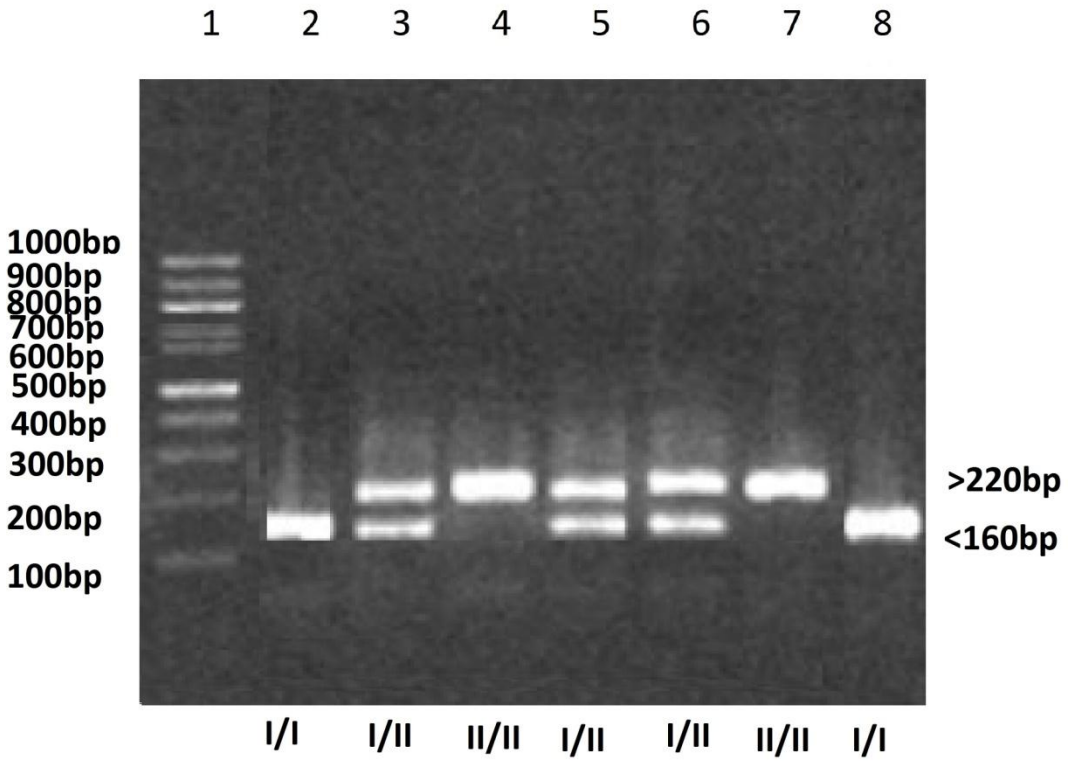
Annealing - 54^0C – 30sec

Extension - 72^0C – 1min

Final extension at 72^0C - 10 min.

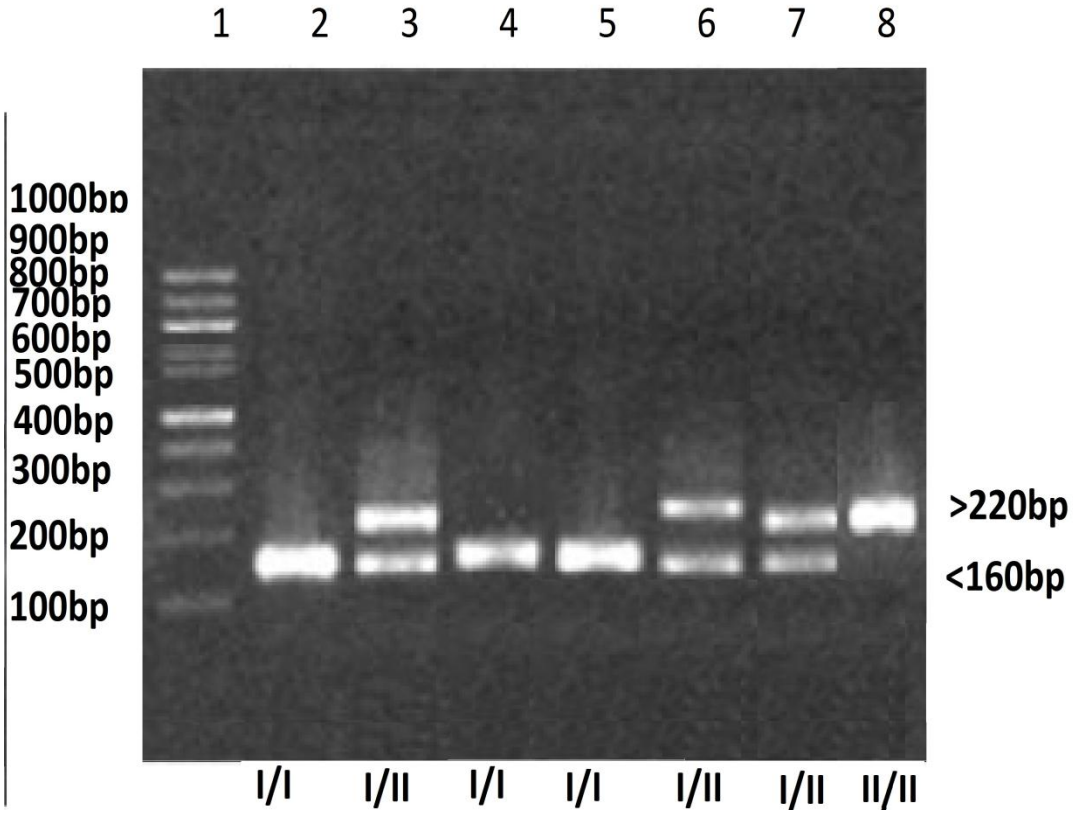
* Amplified product – amplicons was identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder. (Figure:21)

FIGURE – 21



* Amplified products are grouped into two classes. PCR products < 160 bp were grouped as Class I allele and PCR products >220bp were grouped as Class II allele. These PCR products were identified by 2% agarose gel electrophoresis by comparison with a known 100bp DNA ladder. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: bands <160bp(I/I genotype), bands <160 and >220 bp (I/II genotype), or bands >220bp (II/II genotype) (Figure .22)

FIGURE – 22



AGAROSE GEL ELECTROPHORESIS

* PCR product is run on agarose gel in a 100 mL agarose cast as follows: 2g of agarose is weighed and dissolved in 100mL of TAE buffer with a pH of 8.0.

* It is microwaved for 2 mins, cooled and 5 µL of ethidium bromide (10mg/mL) is added. It is poured into a cast and allowed to solidify for 30 min before it is kept in the electrophoresis tank.

* 12 µL of PCR product is loaded onto wells and 4 µL of 100bp DNA ladder is loaded onto single well as a marker. It is electrophoresed at 8V/cm for 45min and visualized under UV illumination.

The biochemical parameters undertaken for the study were determined using the following methodologies:

Estimation of Fasting plasma glucose:

Method: Glucose oxidase peroxidase (GOD/POD), Enzymatic method

Principle:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenolic compound} + 4\text{aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Pink coloured complex} + 2H_2O$$

The intensity of pink coloured compound is directly related to amount of glucose and the OD was measured at 505nm.

Procedure:

To 1 ml of working reagent, 10 µL of plasma was added and incubation done at 37°C-fifteen minutes and OD was read at 505nm.

Reference range :

Fasting plasma glucose - → 70 - 100 mg/dL

Estimation of Blood Urea:

Method : GLDH method, Enzymatic method

Principle:

The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4 + CO_2$$

$$NH_4 + NADH + H^+ + \text{2-Oxoglutarate} \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+$$

The initial rate of reduction in OD at 340 nm is directly related to the concentration of urea.

Procedure:

To 1 mL of working reagent 10 µL of sample or standard is added. Absorbance is measured after 30 sec (A1) and 90 sec (A2).

Estimation :

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times 50 \text{ (Concentration of standard)} = \text{mg/dL of urea}$$

Reference range:

Normal blood urea = 15 to 40 mg/dL

Estimation of Serum Creatinine:

Method : Modified Jaffe's Method.

Principle:

Creatinine complexes with alkaline picrate to give an orange-yellow coloured creatinine picrate. The intensity of this colour is directly proportional to creatinine concentration and is detected at 520 nm by spectrophotometer.

Procedure:

To 500 mL of working reagent 50 µL of serum sample or standard is added and initial absorbance (A1) is measured at 20 sec and final absorbance (A2) is measured at 80 sec after mixing.

Calculation:

$\Delta A = A_2 - A_2$

$$\text{Creatinine (mg/dL)} = \frac{\Delta A \text{ of Test}}{\Delta A \text{ of Standard}} \times \text{Concentration of Standard (2 mg/dl)}$$

Normal range : 0.6-1.2 mg/dL

LIPID PROFILE

Estimation of Plasma Total Cholesterol

Method: Cholesterol Esterase – Cholesterol Oxidase

Kit used: Autospan of Span Diagnostics Ltd.

Principle :

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4- Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinine} + 4H_2O$$

Red quinine is directly related to the cholesterol level and the OD is read at 500nm.

Reagents :

Reagent 1 (Enzymes / Chromogen)

Cholesterol Esterase	≥ 200U/L
Cholesterol Oxidase	≥ 250U/L
Peroxidase	≥ 1000 U/L
4- Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	50mmol/L
Phenol	25mmol/L
Sodium Cholate	0.5 mmol/L

Standard (Cholesterol = 200mg/dL)

Cholesterol	2g/L

Procedure :

To 1 mL of the reconstituted reagent, 10 µL of plasma is added and reading is taken after 5 mins of incubation at 37^{o} C.

Reference Values :

Cholesterol : 150-260 mg /dL

Estimation of Plasma Triglyceride

Method :

Enzymatic Colorimetric method

Kit Used :

Autopak of Bayer Diagnostics

Principle :

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol -3- Phosphate} + ADP$$

$$\text{Glycerol -3- Phosphate} \xrightarrow{\text{Glycerol -3- Phosphate Oxidase}} \text{Dihydroxyacetonephosphate} + H_2O_2$$

$$2\ H_2O_2 + 4\ \text{Aminoantipyrine} + \text{ADPS} \xrightarrow{\text{Peroxidase}} \text{Red Quinone} + H_2O$$

The intensity of coloured complex formed during the reaction is directly proportional to the triglyceride concentration and is estimated at 546nm.

Reagents :

Reagent 1 (Enzymes / Chromogen) :

Lipoprotein Lipase	≥ 1100U/L
Glycerol Kinase	≥ 800U/L
Glycerol -3- Phosphate Oxidase	≥ 5000 U/L
Peroxidase	≥ 350 U/L
4- Aminoantipyrine	0.7 mmol/L
ATP	0.3 mmol/L

Reagent 1A (Buffer) :

Pipes buffer. pH 7.50	50mmol/L
ADPS	1mmol/L
Magnesium salt	15 mmol/L

Standard (Triglycerides 200mg / dL) :

Glycerol (Trig.Equivalent)	2g/L

Reagent Reconstitution:

Reagents were brought to room temperature. Contents of one bottle of reagent 1 were dissolved with one bottle of reagent 1A. It was mixed gently by swirling.

Procedure :

To 1 mL of the reconstituted reagent 10 µL of plasma is added and read at 546nm after incubation at 37^{o}C for 5mins.

Reference Range :

Males	60- 165 mg/dL
Females	40- 140 mg/dL

Estimation of HDL Cholesterol

Method : Immunoinhibition

Kit used : Erba XL System Packs

Principle :

Chylomicrons, VLDL, and LDL fractions in plasma are separated from HDL by immunoinhibition. This is achieved by complexing lipoproteins (Chylomicrons, VLDL, and LDL) other than HDL with Anti human β-lipo protein antibody present in the reagent 1. When reagent 2 is added these complexes inhibits the enzyme reactions. Cholesterol esterase and cholesterol oxidase in reagent 2 react only with HDL-C. Hydrogen peroxide combines with F-DAOS and 4-aminoantipyrine (4-AA) in the presence of peroxidase(POD) and this gives a blue coloured complex. The strength of the colour produced measured at 593 nm is directly related to the HDL-C.

$$\text{LDL, VLDL \& Chylomicrons} \xrightarrow{\text{Anti-LipoproteinB antibody}} \text{antigen antibody complex}$$

$$\text{HDL-C} + H_2O + O_2 \xrightarrow{\text{CHE \& CO}} \text{4-cholestenone} + \text{Fatty acid} + H_2O_2$$

$$H_2O_2 + \text{DAOS} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Blue colored complex} + 2H_2O$$

Reagents :

Reagent 1 :

Goods buffer pH 7.0 30.0mmol/L

4-AAP 0.9mmol/L

POD 2400U/L

Ascorbate oxidase 2700U/L

Antihuman β lipoprotein antibody

Reagent 2 :

Goods buffer, pH – 7.0 30.0mmol/L

CHE 4000U/L

CO 20000U/L

F-DAOS 0.8mmol/L

Calibrator :

HDL-C 56.5mg/dL

Procedure :

Reagent 1 & 2 are mixed in the ratio of 3:1 or 1 bottle of reagent 1 is mixed with 1 bottle of reagent 2 and placed in the auto analyser with the following assay parameters:

Assay type : 2 point

Primary wavelength nm: 600, Secondary wavelength nm : 700

R-1 volume : 270, R-2 volume : 90

Reaction direction : increasing, Sample volume : 3 µL

Calibration : straight

Reference Values :

Adult male : 35.3 – 79.5 mg /dL

Adult female : 42.0 – 88.0 mg / dL

VLDL and LDL Cholesterol

These parameters were calculated using Friedwald's formula given below:

LDL-C = TC-(HDL-C+ VLDL-C)

VLDL-C = TGL/5

Estimation of Serum Leptin levels – ELISA method

Samples:

In the present study serum samples were stored at -20^{o}c and analysed within 3 months.

Human Leptin Assay by ELISA:

Principle:

This enzyme immunoassay test is based on 2-step capture / sandwich ELISA. 2 very specific MABs are used:

One leptin-specific MAB is used

Other MAB is specific for a different epitope of leptin which is conjugated to biotin.

The monoclonal antibody specific for leptin is immobilized onto the the microwell plate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin.

Step 1: Leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step.

Step 2: Streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step.

Step 3: The enzyme substrate TMB is added, forming a blue coloured product that is directly proportional to the amount of leptin present.

Step 4: The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour.

The absorbance is measured on a microtiter plate reader at 450nm.

A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

Reagents:

Human leptin ELISA kit was procured from dbc-Diagnostics Biochem Canada Inc.. Each kit has 96 microtiter well plate and contains the following reagents.

1. One 96 well (12*8) antileptin monoclonal antibody coated microwell plate- break apart wells.

2. Monoclonal anti-leptin antibody conjugated to biotin in a protein-based buffer with a non mercury preservative.

3. Streptavidin- HRP Conjugate in a protein-based buffer with a non mercury preservative 0.4mL/vial. Preparation: Dilute 1:50 in assay buffer before use (eg. 40µL of concentrate in 2 mL of assay buffer. Preparation: dilute 1:50 in assay buffer before use (eg.40 µL of concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of concentrate in 12 mL of assay buffer.

4. Leptin calibrators-

Contents: Six vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin.

Calibrator	Concentration	Volume/vial
Calibrator A	0 ng/mL	0.5 mL
Calibrator B	1 ng/mL	0.5 mL
Calibrator C	5 ng/mL	0.5 mL
Calibrator D	10 ng/ mL	0.5 mL
Calibrator E	20 ng/ mL	0.5 mL
Calibrator F	50 NG/ mL	0.5 mL
Calibrator G	100 ng/ mL	0.5 mL

5. Controls

Contents: two vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of leptin

6. Wash buffer concentrate

Contents: Buffer with a non-ionic detergent and non-mercury preservative.

Preparation: dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

7. Assay buffer

Contents: A protein- based buffer with a non- mercury preservative.

8. TMB Subtrate

Contents: Tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

9. Stopping solution

Contents: 1M sulfuric acid.

Storage and stability: All reagents were refrigerated at 2-8°C

Assay procedure :

All reagents must reach room temperature before use.

1. Prepare working solutions of the streptavidin-HRP conjugate and wash buffer.
2. Pipette 20µL of each calibrator, control and serum sample into correspondingly labelled wells in duplicate.
3. Pipette 80µL of the monoclonal anti-leptin-biotin conjugate into each well.

4. Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
5. Wash the wells 3 times with prepared wash buffer(300 µL/ well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry.
6. Pipette 100 µL of prepared streptavidin-HRP conjugate into each well.
7. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
8. Wash the wells again in the same manner as step 5.
9. Pipette 100µL of TMB substrate into each well at timed intervals.
10. Incubate on a plate shaker for 10 -15 minutes at room temperature.
11. Pipette 50µL of stopping solution into each well at the same timed intervals as in step 9.
12. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

CALCULATIONS :

1. Calculate the mean optical density of each calibrator.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the y-axis and the calibrator concentrations on the x-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.

3. Calculate the mean optical density of each unknown sample.

4. Read the values of the unknowns directly off the calibrator curve.

5. If a sample reads more than 100 ng/mL then dilute it with assay buffer at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

Typical tabulated Data:

Calibrator	OD1	OD2	Mean OD	Value (ng/mL)
A	0.073	0.070	0.072	0
B	0.102	0.100	0.101	1
C	0.290	0.293	0.292	5
D	0.620	0.630	0.625	10
E	1.140	1.086	1.113	20
F	1.947	1.919	1.933	50
G	2.518	2.514	2.516	100
Unknown	0.275	0.273	0.274	422

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

GROUP	**MEAN (ng/ mL)**	**RANGE (ng/ mL)**
Lean Women	7.4	3.7-11.1
Lean Men	3.8	2.0-5.6

Leptin values are approximately 2.5 times higher in women than men per unit BMI.

Human insulin assay by ELISA:

The calbiotech Insulin ELISA Kit was used for the quantitative measurement of Insulin in human serum.

PRINCIPLE OF THE TEST

The insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with the enzyme (HRP)-conjugated anti-insulin antibody and anti-insulin antibody bound to micro-titration well. A simple washing step removes unbound enzyme labeled antibody. The bound HRP complex is detected by reaction with TMB substrate. The reaction is stopped by adding acid to give a colorimetric endpoint that is read using ELISA reader.

MATERIALS PROVIDED

1. Microwell coated with Insulin MAb
2. Insulin Standard 1-6
3. Insulin Enzyme Conjugate
4. Assay Diluent
5. TMB Substrate

6. Stop Sloution

7. 20X Wash concentrate

Storage and Stability

All reagents were stored at 2-8° C.

SPECIMEN COLLECTION HANDLING

1. Blood specimens were collected and the serum was separated immediately.
2. Specimens were stored frozen at (-20°C) for up to one month.
3. Frozen sera were completely thawed and mixed well before estimation.

REAGENT PRPARATION

1. 20X Enzyme Conjugate: 1x working dilution at 1:20 with assay diluents was prepared eg, 0.1 ml of the stock conjugate in 1.9 ml of assay diluents is sufficient for 20 wells. The diluted conjugate was used on the same day.
2. 20X Wash Buffer Concentrate: 1X wash buffer was prepared by adding the contents of the bottle to 475 ml of distilled water.

ASSAY PROCEDURE

Prior to assay regeants were allowed to stand at room temperature.

Regeants were gently mixed before use.

1. Place the desired number of coated strips into the holder.

2. Pipette 25 µl of insulin standards, control and patient's sera into appropriate wells.
3. Add 100 µl of working Insulin Enzyme Conjugate to all wells.
4. Thoroughly mix for 10 sec, it is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature(18-26° C)
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100µl of stop TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows.

1. Check insulin standard value on each standard vial.
2. To construct the standard curve, plot the absorbance for the insulin standards (vertical axis) versus the insulin standard concentrations in µIU/mL (horizontal axis) on the linear graph paper. Draw the best curve through the points.

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard are related after diluting with “0” standard.

EXPECTED VALUES

The expected values of serum insulin using this ELISA kit was <25 µIU/mL .

HOMA-IR:

HOMA-IR was determined to measure the insulin resistance .

HOMA IR= (fasting serum insulin in µU/mL x fasting plasma glucose in mmol/L)/22.5

CALCULATION OF BMI:

Body mass index was determined by the following formula.

BMI = Weight in kgs / Height in m^2

Statistical Analysis

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software version 17.0

1. Descriptive statistics was derived for all the parameters given in the master chart for both cases and controls.
2. Comparision was made using Chi-square test and Independent samples t-test to find if there were any difference in all the parameters among hypertensives and apparently healthy controls. P value taken as significant is less than 0.05.
3. Distribution and frequencies of the genotypes between cases and controls were compared with a χ^2 test.
4. The allele frequencies (Class I &Class II) among cases and controls were calculated by allele counting.
5. Chi square test was used to compare the allele proportions between cases and controls for 2*2 contingency table.
6. Associated risk of essential hypertension and classI/classI genotype was calculated by using Odds ratio.

7. Odds ratio was adjusted for BMI to know whether the association of LEP-tet polymorphism was independent of obesity.
8. To know the relation between genotypes and serum leptin levels among hypertensives and apparently healthy controls ANOVA was used.
9. Comparision between the insulin levels and mean HOMA-IR index among different genotypes was done with ANOVA
10. ANOVA was used to compare the relation between the genotypes and BMI among hypertensives and apparently healthy controls.
11. Pearsons correlation test was used to detect the relation between serum leptin levels and HOMA-IR among cases and controls.
12. To determine if there is any relation of leptin concentrations to BMI among hypertensives and apparently healthy controls, Pearson correlation test was used.
13. Correlation of concentrations of leptin to lipid parameters among the cases and controls were determined using Pearson correlation analysis .
14. Linear regression analysis was done to determine any significant association between leptin and other confounding parameters like age,gender,BMI,smoking, alcohol and genotypes.
15. Multiple logistic regression was carried out to determine any significant association of leptin gene polymorphism with essential hypertension after keeping the influence of the confounding variables as constant.

16. Hardy-Weinberg principle calculation was done to check whether the genotypes of the study population was in Hardy-Weinberg equilibrium.

Results

MASTER CHART FOR CASES-HYPERTENSIVES

S.no	Age	Sex	Sys BP	Dia BP	BMI	Smoking	Alcohol	Sugar	Urea	creatinine	TGL	cholesterol	HDL	LDL	LEP GEN OTYPE	Leptin	Insulin	HOMA-IR
1	65	M	130	90	19.96	Y	Y	86	40	1.2	99	200	30	150.2	I/II	0.71	0.071	0.02
2	53	M	150	100	19.23	Y	Y	82	28	0.7	76	180	40	124.8	I/II	2.4	0.07	0.01
3	48	M	140	100	20.57	Y	Y	89	34	0.9	121	186	35	126.8	I/I	1.4	11.3	2.48
4	52	M	160	100	21.19	N	Y	80	20	0.5	100	164	34	110	II/II	13.61	100.4	19.83
5	48	M	140	100	22.31	N	Y	89	34	0.9	69	192	41	137.2	I/II	14.4	2.18	0.48
6	45	M	160	100	21.51	N	N	102	42	1.3	71	208	39	154.8	I/I	6.1	42	10.58
7	50	F	146	90	22.83	N	N	100	36	1	90	198	41	139	II/II	48.9	13.98	3.45
8	45	M	140	90	21.77	N	N	76	24	0.6	88	164	38	108.4	I/II	1.67	0.14	0.03
9	52	F	160	100	21.93	N	N	86	38	1.1	82	200	38	145.6	I/II	38	7.74	1.64
10	55	F	146	90	21.36	N	N	100	38	1.1	105	152	45	86	II/II	57.43	2.22	0.55
11	60	M	180	110	22.66	N	N	94	40	1.2	119	163	35	104.2	I/I	7.2	3.15	0.73
12	53	M	170	130	24.38	N	Y	89	24	0.6	130	178	33	119	I/II	6.9	7.92	1.74
13	47	M	146	90	24.14	N	N	86	34	0.9	68	198	42	142.4	I/I	5.8	1.13	0.24
14	46	M	180	110	23.99	N	N	88	32	0.8	89	182	37	127.2	II/II	2.3	35.17	7.64
15	56	M	160	110	24.77	N	N	99	38	1.1	92	204	38	147.6	I/II	4.02	11.51	2.81
16	57	M	150	100	24.77	Y	Y	90	34	0.9	71	194	43	136.8	I/II	6.5	4.24	0.94
17	60	M	140	90	23.59	N	N	88	40	1.2	74	192	40	137.2	I/II	8.6	4.31	0.94
18	55	M	150	100	24.03	Y	Y	95	37	1	96	198	43	135.8	II/II	6.1	0.93	0.22
19	50	M	160	90	24.98	N	N	100	36	1	79	195	37	142.2	I/I	0.94	53.81	13.29
20	47	M	170	100	23.42	Y	N	80	34	0.9	76	194	41	137.8	I/II	5.05	1.12	0.22
21	48	M	140	100	23.05	N	N	85	34	0.9	89	194	43	133.2	II/II	4.8	0.61	0.13
22	47	M	160	110	23.12	N	N	80	24	0.6	280	170	32	82	I/II	2.6	0.27	0.05
23	45	M	170	100	24.69	N	N	88	30	0.8	176	192	33	123.8	II/II	5.1	8.61	1.87
24	49	M	140	100	25.39	Y	N	92	32	0.9	86	195	41	136.8	II/II	10.3	0.11	0.02
25	64	F	140	90	26.28	N	N	74	40	1.2	85	208	48	143	I/I	81.8	1.03	0.19
26	47	M	130	100	25.95	Y	Y	92	31	0.8	77	190	41	133.6	I/II	45.4	11.2	2.54
27	51	M	146	110	27.44	Y	Y	85	28	0.7	92	182	42	121.6	II/II	14	0.37	0.08
28	53	M	140	100	28.95	Y	Y	80	28	0.7	78	195	38	141.4	II/II	9.6	0.77	0.15
29	58	F	140	100	29	N	N	94	38	1.1	98	198	40	138.4	I/II	38.4	40.55	9.41
30	60	F	160	120	29.52	N	N	100	40	1.2	102	200	37	142.6	II/II	50.6	22.46	5.55
31	59	M	160	120	25.71	N	N	87	33	0.8	83	180	41	122.4	II/II	3.4	8.07	1.73
32	58	M	180	11O	26.14	Y	Y	97	36	0.9	160	174	38	104	I/I	51.1	1.12	0.27
33	50	F	150	110	27.01	N	N	100	32	1	171	196	35	126.8	II/II	42.6	14.17	3.50
34	45	F	190	120	27.03	N	N	90	36	1	96	192	43	129.8	II/II	52.2	0.71	0.16
35	53	F	150	90	25.78	N	N	84	30	0.8	114	182	31	128.2	I/II	50.3	6.18	1.28
36	46	M	150	100	25.59	Y	N	93	32	0.9	118	173	34	115.4	II/II	16.7	7.66	1.76
37	48	F	110	80	27.39	N	N	70	26	0.5	77	182	39	127.6	I/I	51.4	5.65	0.98
38	46	M	150	100	27.85	N	N	86	36	1	82	198	40	141.6	I/II	15.7	10.9	2.31

39	65	F	170	100	28.4	N	N	74	28	0.7	84	204	43	144.2	II/II	70.2	0.16	0.03
40	39	F	140	90	29.38	N	N	84	34	0.8	161	199	37	129.8	II/II	50.3	0.24	0.05
41	57	F	140	100	29.34	N	N	90	32	0.7	205	190	33	116	I/II	38.6	4.99	1.11
42	50	M	140	100	28.2	N	N	82	29	0.6	172	192	32	125.6	I/I	4.4	5.2	1.05
43	54	M	130	90	25.1	N	N	89	34	0.9	84	194	42	135.2	II/II	5.9	9.36	2.06
44	55	M	148	96	27.27	Y	N	82	27	0.8	190	188	32	118	II/II	9.4	1.65	0.33
45	48	F	150	100	35.18	N	N	83	29	0.9	165	194	43	118	I/II	65.9	11.98	2.46
46	45	F	160	110	33.16	N	N	100	32	0.9	92	195	40	136.6	I/II	41.2	58.7	14.49
47	58	F	160	100	34.24	N	N	100	38	1.1	211	204	31	130.8	I/II	63.5	6.25	1.54
48	46	F	140	90	32.43	N	N	86	32	0.8	176	186	35	115.8	I/II	79.1	1.27	0.27
49	46	M	140	90	20.94	N	N	80	32	0.8	110	189	34	133	I/I	7.9	5.76	1.14
50	56	F	148	90	32.39	N	N	97	36	1	120	198	36	138	II/II	90.9	52.87	12.66
51	57	F	160	110	31.58	N	N	98	38	1.1	107	206	36	148.6	II/II	131.5	16.39	3.97
52	47	M	140	90	30.12	N	N	88	32	0.8	102	187	38	128.6	I/I	21.1	1.11	0.24
53	57	F	160	80	32.79	N	N	101	38	1.1	89	198	42	138.2	I/II	99.9	51.45	12.83
54	51	F	140	100	31.63	N	N	100	36	1	67	196	44	138.6	II/II	113	19.67	4.86
55	52	F	140	90	31.2	N	N	89	28	0.7	98	182	43	119.4	I/I	103.5	7.42	1.63
56	51	F	140	100	22.83	N	N	76	23	0.6	151	137	32	74.8	I/II	15.5	6.87	1.29
57	49	F	150	90	21.36	N	N	80	24	0.7	88	123	46	59.4	II/II	30.35	4.31	0.85
58	50	M	146	96	22.31	Y	N	94	28	0.9	115	135	35	77	I/II	8.3	1.62	0.38
59	45	M	150	90	22.66	Y	Y	79	36	1	73	111	44	52.4	I/II	45.7	9.82	1.92
60	61	M	152	94	23.4	Y	N	96	28	0.9	88	198	38	142.4	II/II	38.4	40.55	9.61
61	52	F	160	90	22.31	N	N	82	24	0.7	90	189	39	132	I/I			
62	48	F	146	100	29	N	N	94	38	1.1	98	198	40	138.4	II/II			
63	60	F	156	90	29.52	N	N	100	40	1.2	102	200	37	142.6	I/II			
64	69	M	148	100	25.71	N	N	87	33	0.8	83	180	41	122.4	I/II			
65	58	M	160	90	26.14	Y	Y	97	36	0.9	160	174	38	104	II/II			
66	52	F	150	90	27.01	N	N	100	32	1	171	196	35	126.8	II/II			
67	45	F	160	90	27.03	N	N	90	36	1	96	192	43	129.8	I/II			
68	63	F	140	90	25.78	N	N	84	30	0.8	114	182	31	128.2	II/II			
69	56	M	150	90	25.59	Y	N	93	32	0.9	118	173	34	115.4	II/II			
70	68	F	140	100	27.39	N	N	70	26	0.5	77	182	39	127.6	I/II			
71	46	M	150	90	27.85	N	N	86	36	1	82	198	40	141.6	I/II			
72	65	F	140	90	28.4	N	N	74	28	0.7	84	204	43	144.2	I/I			
73	49	F	150	90	29.38	N	N	84	34	0.8	161	199	37	129.8	II/II			
74	57	F	150	100	29.34	N	N	90	32	0.7	205	190	33	116	II/II			
75	50	M	140	90	28.2	N	N	82	29	0.6	172	192	32	125.6	I/II			
76	54	M	142	92	25.1	N	N	89	34	0.9	84	194	42	135.2	I/II			
77	65	M	158	96	27.27	Y	N	82	27	0.8	190	188	32	118	II/II			
78	48	F	150	98	35.18	N	N	83	29	0.9	165	194	43	118	I/II			
79	45	F	156	92	33.16	N	N	100	32	0.9	92	195	40	136.6	II/II			
80	59	F	140	94	34.24	N	N	100	38	1.1	211	204	31	130.8	I/II			
81	46	F	144	92	32.43	N	N	86	32	0.8	176	186	35	115.8	II/II			
82	46	M	150	90	20.94	N	N	80	32	0.8	110	189	34	133	II/II			
83	56	F	146	96	32.39	N	N	97	36	1	120	198	36	138	I/I			

84	57	F	152	100	31.58	N	N	98	38	1.1	107	206	36	148.6	I/II			
85	47	M	140	94	30.12	N	N	88	32	0.8	102	187	38	128.6	I/II			
86	57	F	150	90	32.79	N	N	101	38	1.1	89	198	42	138.2	II/II			
87	51	F	142	90	31.63	N	N	100	36	1	67	196	44	138.6	I/II			
88	52	F	150	90	31.2	N	N	89	28	0.7	98	182	43	119.4	II/II			
89	61	F	144	100	22.83	N	N	76	23	0.6	151	137	32	74.8	I/II			
90	49	F	148	90	21.36	N	N	80	24	0.7	88	123	46	59.4	I/II			
91	50	M	140	98	22.31	Y	N	94	28	0.9	115	135	35	77	II/II			
92	45	M	150	92	22.66	Y	Y	79	36	1	73	111	44	52.4	I/I			
93	61	M	156	92	23.4	Y	N	96	28	0.9	88	198	38	142.4	I/II			
94	45	F	156	90	22.31	N	N	82	24	0.7	98	188	38	130.4	I/II			
95	50	F	148	90	22.83	N	N	100	36	1	90	198	41	139	II/II			
96	58	M	160	90	21.77	N	N	76	24	0.6	88	164	38	108.4	I/II			
97	53	F	162	100	21.93	N	N	86	38	1.1	82	200	38	145.6	I/II			
98	55	F	142	92	21.36	N	N	100	38	1.1	105	152	45	86	I/II			
99	56	M	158	100	22.66	N	N	94	40	1.2	119	163	35	104.2	I/I			
100	61	M	160	96	24.38	N	Y	89	24	0.6	130	178	33	119	I/II			

MASTER CHART FOR HEALTHY CONTROLS																		
S.no	**Age**	**Sex**	**Sys BP**	**Dia BP**	**BMI**	**Smoking**	**Alcohol**	**Sugar**	**Urea**	**creatinine**	**TGL**	**cholesterol**	**HDL**	**LDL**	**LEP GENOTYPE**	**Leptin**	**Insulin**	**HOMA-IR**
1	30	M	120	80	18.51	Y	N	78	26	0.7	105	172	31	120	I/II	0.9	0.29	0.06
2	49	M	120	80	19.53	N	Y	86	34	0.9	110	196	36	138	I/II	0.73	0.69	0.15
3	62	M	110	70	22.19	N	N	82	38	1.1	121	206	32	149.8	I/II	1.8	14.67	2.97
4	62	F	110	70	18.9	N	N	98	28	1	83	204	42	145.4	II/II	5.6	0.01	0.00
5	52	M	100	70	20.24	Y	N	88	36	0.8	118	198	37	137.4	I/II	2.1	0.17	0.04
6	37	M	110	70	19.95	Y	N	78	24	0.7	65	144	39	92	II/II	0.8	0.27	0.05
7	43	F	110	70	17.12	N	N	98	34	0.9	115	196	37	136	I/II	4.5	6.95	1.68
8	40	F	120	80	22.18	N	N	79	32	0.6	140	192	38	126	II/II	3.9	4.85	0.95
9	48	M	120	70	22.99	Y	Y	90	30	1	225	198	33	120	II/II	2.7	0.66	0.15
10	35	F	120	70	22.03	N	N	92	32	0.8	113	184	34	127.4	II/II	5.5	0.89	0.20
11	43	F	110	70	19.98	N	N	90	33	0.9	80	192	39	137	II/II	4.3	7.07	1.57
12	50	F	120	70	20	N	N	94	26	0.5	68	137	42	81.4	II/II	10.8	0.88	0.20
13	36	M	120	70	22.94	N	N	90	30	0.8	103	188	32	135.4	I/II	5.1	0.78	0.17
14	52	M	120	80	17.09	Y	N	96	31	0.9	139	192	35	129.2	II/II	2.6	1.55	0.37
15	32	F	110	70	20	N	N	98	27	0.7	124	182	36	121.2	I/I	10.9	12.79	3.09
16	45	F	110	70	18.59	N	N	98	28	0.8	97	159	42	97.6	I/II	2.2	1.08	0.26
17	45	M	110	70	23.28	N	N	79	29	0.9	100	194	31	143	II/II	3.9	9.34	1.82
18	38	M	120	80	26.08	Y	Y	86	29	0.8	84	227	44	166.2	II/II	6.1	0.73	0.16
19	47	M	120	80	26.71	Y	N	76	25	0.6	70	196	47	135	II/II	5.2	1.74	0.33
20	47	M	130	80	25.1	N	Y	80	24	0.6	169	174	33	107.2	I/II	2.3	1.42	0.28
21	43	F	120	80	24.34	N	N	94	32	0.8	116	199	34	141.8	II/II	25	0.73	0.17
22	45	M	120	80	25.61	Y	N	90	34	0.7	72	222	45	162.6	I/II	14.7	10.69	2.38
23	34	M	100	70	25.1	Y	N	92	28	0.9	174	189	33	121.2	II/II	10.8	11.98	2.72
24	39	F	120	80	26.67	N	N	100	36	0.7	83	185	39	129.4	II/II	60.7	1.11	0.27
25	52	F	120	80	29.64	N	N	98	36	1	88	198	42	138.4	I/II	79.4	40.11	9.71
26	44	F	120	70	28.76	N	N	92	34	0.9	60	192	39	141	II/II	63.3	8.61	1.96
27	46	F	120	80	24.89	N	N	94	36	1	66	186	41	131.8	I/II	66.5	7.7	1.79
28	47	M	120	70	25.15	Y	Y	90	34	0.9	132	192	35	130.6	II/II	0.7	4.96	1.10
29	48	M	110	70	38.54	Y	N	88	32	0.8	80	198	39	143	II/II	82.5	28.4	6.17
30	56	M	100	70	25.61	Y	N	100	38	0.9	164	199	36	130.2	I/I	7.8	10.98	2.71
31	60	M	120	70	22.03	N	N	86	38	1.1	105	172	31	120	II/II			
32	55	M	110	70	23.28	N	N	100	40	1.2	110	196	36	138	II/II			
33	50	M	120	70	24.34	N	N	94	33	0.8	121	206	32	149.8	I/II			
34	47	M	120	70	17.09	N	N	89	36	0.9	83	204	42	145.4	II/II			
35	48	M	100	70	26.08	N	N	86	32	1	118	198	37	137.4	II/II			
36	47	M	120	80	22.94	N	N	88	36	1	65	144	39	92	II/II			
37	45	F	120	80	19.53	N	N	99	30	0.8	115	196	37	136	II/II			
38	49	M	120	80	26.08	N	N	90	32	0.9	140	192	38	126	I/II			
39	64	F	120	70	18.9	N	N	88	26	0.5	225	198	33	120	II/II			

40	47	F	110	70	20.24	N	N	95	36	1	113	184	34	127.4	I/II		
41	51	M	100	70	19.95	N	N	100	28	0.7	80	192	39	137	II/II		
42	53	M	120	80	23.28	Y	N	80	34	0.8	68	137	42	81.4	I/II		
43	58	M	120	80	17.09	Y	Y	85	32	0.7	103	188	32	135.4	II/II		
44	60	M	110	70	22.99	Y	N	80	29	0.6	139	192	35	129.2	II/II		
45	59	M	110	70	25.15	N	N	88	34	0.9	124	182	36	121.2	II/II		
46	58	M	100	70	22.19	N	N	92	27	0.8	97	159	42	97.6	II/II		
47	50	M	110	70	22.03	N	N	74	29	0.9	300	194	31	103	II/II		
48	45	M	110	70	19.98	N	N	92	32	0.9	84	227	44	166.2	I/II		
49	53	M	120	80	26.08	N	N	85	38	1.1	70	196	47	135	II/II		
50	46	M	120	70	22.94	N	N	80	32	0.8	169	174	33	107.2	II/II		
51	48	M	110	70	17.09	N	Y	94	32	0.8	116	199	34	141.8	II/II		
52	46	M	100	70	20	N	N	100	36	1	72	222	45	162.6	I/II		
53	65	M	120	80	18.59	N	N	87	38	1.1	174	189	33	121.2	II/II		
54	39	M	120	80	23.28	Y	N	97	32	0.8	83	185	39	129.4	I/II		
55	57	F	120	80	23.28	N	N	100	38	1.1	88	198	42	138.4	II/II		
56	50	M	120	80	18.9	N	N	90	36	1	60	192	39	141	II/II		
57	54	M	130	80	25.1	N	N	84	28	0.7	66	186	41	131.8	I/II		
58	55	M	120	80	24.34	N	N	93	23	0.6	132	192	35	130.6	II/II		
59	48	F	120	80	25.61	N	N	70	24	0.7	80	198	39	143	II/II		
60	45	F	100	70	25.1	N	N	86	32	0.6	164	199	36	130.2	I/II		
61	58	M	120	80	26.67	N	N	74	30	1	107	206	36	148.6	I/II		
62	46	M	120	80	29.64	N	N	84	32	0.8	102	187	38	128.6	II/II		
63	46	F	120	70	28.76	N	N	90	33	0.9	89	198	42	138.2	II/II		
64	56	F	120	80	24.89	N	N	89	26	0.5	67	196	44	138.6	I/II		
65	57	F	120	70	25.15	N	N	76	30	0.8	98	182	43	119.4	II/II		
66	47	M	110	70	38.54	N	N	80	31	0.9	151	137	32	74.8	II/II		
67	57	F	100	70	22.94	N	N	94	27	0.7	88	123	46	59.4	II/II		
68	51	M	100	70	22.03	Y	N	79	28	0.8	115	135	35	77	II/II		
69	52	F	110	70	23.28	N	N	96	29	0.9	73	111	44	52.4	I/II		
70	51	F	110	70	22.19	N	N	82	29	0.8	88	198	38	142.4	II/II		
71	49	F	120	80	38.54	N	N	94	34	0.9	98	188	38	130.4	II/II		
72	50	M	120	70	25.61	N	N	100	32	0.8	90	198	41	139	II/II		
73	45	M	120	70	22.03	N	N	87	38	0.9	88	164	38	108.4	I/II		
74	61	M	110	70	23.28	N	N	97	38	1.1	82	200	38	145.6	II/II		
75	52	F	120	70	24.34	N	N	100	40	1.2	105	152	45	86	II/II		
76	48	F	120	70	23.28	N	N	90	34	0.9	119	163	35	104.2	II/II		
77	60	F	110	70	26.08	N	N	84	36	0.8	130	178	33	119	II/II		
78	69	F	120	80	22.94	N	N	93	24	0.7	89	198	42	138.2	I/II		
79	58	M	120	80	19.53	N	Y	70	34	0.9	67	196	44	138.6	I/II		
80	52	F	130	80	26.08	N	N	86	32	0.6	98	182	43	119.4	II/II		
81	45	F	120	70	18.9	N	N	74	30	1	151	137	32	74.8	II/II		
82	63	M	110	70	20.24	N	N	84	32	0.8	88	123	46	59.4	II/II		
83	56	F	120	70	19.95	N	N	90	33	0.9	115	135	35	77	I/II		
84	68	F	120	70	23.28	N	N	88	26	0.5	73	111	44	52.4	II/II		

85	46	F	120	80	18.9	N	N	100	30	0.8	88	198	38	142.4	II/II		
86	65	F	110	70	22.99	N	N	86	31	0.9	90	189	39	132	I/II		
87	49	F	110	70	31.63	N	N	100	27	0.7	98	198	40	138.4	II/II		
88	57	M	110	70	31.2	N	N	94	28	0.8	102	200	37	142.6	II/II		
89	50	M	120	80	22.83	N	N	89	29	0.9	83	180	41	122.4	II/II		
90	54	M	120	80	21.36	N	N	86	29	0.8	160	174	38	104	II/II		
91	65	F	130	80	22.31	N	N	88	25	0.6	171	196	35	126.8	I/II		
92	48	F	120	80	22.66	N	N	99	24	0.6	96	192	43	129.8	I/II		
93	45	F	120	80	23.4	N	N	80	32	0.8	114	182	31	128.2	II/II		
94	59	M	100	70	22.31	N	N	85	25	0.6	118	173	34	115.4	II/II		
95	46	M	120	80	22.83	Y	N	80	24	0.6	77	182	39	127.6	II/II		
96	46	F	120	80	21.77	N	N	88	32	0.8	82	198	40	141.6	II/II		
97	56	F	120	70	21.93	N	N	92	34	0.7	84	204	43	144.2	I/II		
98	57	F	120	80	21.36	N	N	74	28	0.9	161	199	37	129.8	II/II		
99	47	M	120	70	25.1	Y	N	92	36	0.7	205	190	33	116	I/I		
100	57	F	120	80	24.34	N	N	85	36	1	172	192	32	125.6	II/II		

TABLE – A

	DESCRIPTIVE STATISTICS	CASES	CONTROLS
AGE	N VALID	100	100
	MISSING	0	0
	MEAN	52.75	50.43
	STD. DEVIATION	6.254	7.846
	MINIMUM	39	30
	MAXIMUM	69	69
SBP	N VALID	100	100
	MISSING	0	0
	MEAN	150.12	115.70
	STD. DEVIATION	11.772	7.420

	MINIMUM	110	100
	MAXIMUM	190	130
DBP	N VALID	100	100
	MISSING	0	0
	MEAN	96.67	74.20
	STD. DEVIATION	8.379	4.960
	MINIMUM	80	70
	MAXIMUM	130	80
BMI	N VALID	100	100
	MISSING	0	0
	MEAN	26.32	23.36
	STD. DEVIATION	4.004	4.084
	MINIMUM	19	17
	MAXIMUM	35	39
SUGAR	N VALID	100	100
	MISSING	0	0
	MEAN	88.82	88.65
	STD. DEVIATION	8.214	7.647
	MINIMUM	70	70
	MAXIMUM	102	100
UREA	N VALID	100	100
	MISSING	0	0
	MEAN	32.44	31.37
	STD. DEVIATION	5.058	4.192
	MINIMUM	20	23

	MAXIMUM	42	40
CREATININE	N VALID	100	100
	MISSING	0	0
	MEAN	.88	.83
	STD. DEVIATION	.186	.158
	MINIMUM	0	0
	MAXIMUM	1	1

	DESCRIPTIVE STATISTICS	**CASES**	**CONTROL**
TGL	N VALID	100	100
	MISSING	0	0
	MEAN	112.58	110.00
	STD. DEVIATION	41.205	40.458
	MINIMUM	67	60
	MAXIMUM	280	300
CHOLESTEROL	N VALID	100	100
	MISSING	0	0
	MEAN	184.62	184.27
	STD. DEVIATION	20.934	23.589
	MINIMUM	111	111
	MAXIMUM	208	227
HDL	N VALID	100	100
	MISSING	0	0
	MEAN	38.23	37.98
	STD. DEVIATION	4.221	4.325
	MINIMUM	30	31

	MAXIMUM	48	47
LDL	N VALID	100	100
	MISSING	0	0
	MEAN	123.87	124.29
	STD. DEVIATION	22.107	23.958
	MINIMUM	52	52
	MAXIMUM	155	166
LEPTIN	N VALID	60	30
	MISSING	40	70
	MEAN	31.58	16.44
	STD. DEVIATION	32.397	25.340
	MINIMUM	1	1
	MAXIMUM	132	82
INSULIN	N VALID	60	30
	MISSING	40	70
	MEAN	12.59	6.40
	STD. DEVIATION	18.985	8.951
	MINIMUM	0	0.01
	MAXIMUM	100	40
HOMAIR	N VALID	60	30
	MISSING	4023.958	70
	MEAN	2.882854E0	1.449002E0
	STD. DEVIATION	4.3154274E0	2.0886774E0
	MINIMUM	.0142	.0024
	MAXIMUM	19.8321	9.7056

TABLE - B

DESCRIPTIVE STATISTICS		CASES			CONTROLS		
		VALID MALE	FEMALE	TOTAL	VALID MALE	FEMALE	TOTAL
SEX	FREQUENCY	51	49	100	56	44	100
	PERCENT	51.0	49.0	100.0	56.0	44.0	100.0
	VALID PERCENT	51.0	49.0	100.0	56.0	44.0	100.0
	CUMULATIVE PERCENT	51.0	100.0		56.0	100.0	

DESCRIPTIVE STATISTICS		CASES			CONTROLS		
		VALID YES	VALID NO	TOTAL	VALID YES	VALID NO	TOTAL
SMOKING	FREQUENCY	22	78	100	19	81	100
	PERCENT	22.0	78.0	100.0	19.0	81.0	100.0
	VALID PERCENT	22.0	78.0	100.0	19.0	81.0	100.0
	CUMULATIVE PERCENT	22.0	100.0		19.0	100.0	

DESCRIPTIVE STATISTICS		CASES			CONTROLS		
		VALID YES	VALID NO	TOTAL	VALID YES	VALID NO	TOTAL
ALCOHOL	FREQUENCY	16	84	100	8	92	100

	PERCENT	16.0	84.0	100.0	8.0	92.0	100.0
	VALID PERCENT	16.0	84.0	100.0	8.0	92.0	100.0
	CUMULATIVE PERCENT	16.0	100.0		8.0	100.0	

DESCRIPTIVE STATISTICS		**CASES**				**CONTROLS**			
LEPTINGENE		**VALID I/ I**	**II/ II**	**I/ II**	**TOTAL**	**VALID I/ I**	**II/ II**	**I/ II**	**TOTAL**
	FREQUENCY	17	39	44	100	3	66	31	100
	PERCENT	17.0	39.0	44.0	100.0	3.0	66.0	31.0	100.0
	VALID PERCENT	17.0	39.0	44.0	100.0	3.0	66.0	31.0	100.0
	CUMULATIVE PERCENT	17.0	56.0	100.0		3.0	69.0	100.0	

TABLE 1

CHI-SQUARE TEST TO COMPARE THE PROPORTIONS BETWEEN CASES AND CONTROLS

Variables	Group				Total		P-Value
	Controls		Cases				
	N	%	N	%	N	%	

LEP GENOTYPE	II/II	66	66.0	39	39.0	105	52.5	<0.001
	I/II	31	31.0	44	44.0	75	37.5	
	I/I	3	3.0	17	17.0	20	10.0	
Gender	Male	56	56.0	51	51.0	107	53.5	0.478
	Female	44	44.0	49	49.0	93	46.5	
Smoking	No	81	81.0	78	78.0	159	79.5	0.599
	Yes	19	19.0	22	22.0	41	20.5	
Alcohol	No	92	92.0	84	84.0	176	88.0	0.082
	Yes	8	8.0	16	16.0	24	12.0	
Total		100	100.0	100	100.0	200	100.0	

TABLE 2

INDEPENDENT SAMPLES T-TEST TO COMPARE THE MEAN VALUES BETWEEN CASES AND CONTROLS

variables	Group	N	Mean	Std. Dev	t-Value	P-Value
Age	Controls	100	50.43	7.846	2.312	0.022
	Cases	100	52.75	6.254		
Sys BP	Controls	100	115.70	7.420	24.736	<0.001
	Cases	100	150.12	11.772		
Dia BP	Controls	100	74.20	4.960	23.081	<0.001
	Cases	100	96.80	8.442		
BMI	Controls	100	23.36	4.084	5.164	<0.001
	Cases	100	26.32	4.004		
Sugar	Controls	100	88.65	7.647	0.151	0.880
	Cases	100	88.82	8.214		
Urea	Controls	100	31.37	4.192	1.629	0.105
	Cases	100	32.44	5.058		
Creatinine	Controls	100	.83	.158	2.379	0.018
	Cases	100	.88	.186		
TGL	Controls	100	110.00	40.458	0.447	0.656
	Cases	100	112.58	41.205		
Cholesterol	Controls	100	184.27	23.589	0.111	0.912
	Cases	100	184.62	20.934		
HDL	Controls	100	37.98	4.325	0.414	0.680
	Cases	100	38.23	4.221		

LDL	Controls	100	124.29	23.958	0.128	0.899
	Cases	100	123.87	22.107		
Leptin	Controls	30	16.44	25.340	2.486	0.015
	Cases	60	31.98	32.520		
Insulin	Controls	30	6.40	8.951	2.123	0.037
	Cases	60	12.69	19.127		
HOMA-IR	Controls	30	1.4490	2.08868	2.153	0.034
	Cases	60	2.9099	4.34664		

TABLE - 3

LEP GENOTYPE	CONTROLS	CASES	P VALUE
II/II	66	39	
I/II	31	44	<0.001
I/I	3	17	

TABLE - 4

CHI-SQUARE TEST TO COMPARE THE ALLELE PROPORTIONS BETWEEN CASES AND CONTROLS

Variables	Group	Total	P-Value

		Controls		Cases				
		N	%	N	%	N	%	
Allele Type	II	163	81.5	122	61.0	285	71.3	<0.001
	I	37	18.5	78	39.0	115	28.7	
Total		200	100.0	200	100.0	400	100.0	

Frequency of Class I allele in hypertensives = 39 %

Frequency of Class I allele in normotensives = 18.5%

TABLE - 5

ASSOCIATION BETWEEN ALLELES AND HYPERTENSION

	CASES	CONTROLS	CASES+CONTROLS
Class I	78	37	115
Class II	122	163	285
I+II	200	200	400

O.R. =2.81657

Wald 95% CI: 1.785 >2.817> 4.445

Chi-square value 20.5156

p-value< 0.0001

TABLE 6(A)

To find an association between CLASS I/ CLASS I genotype and

Essential hypertension.

GENOTYPE		Hypertension		
		Yes	**No**	**Total**
CLASS I/ CLASS I	**Yes**	17	3	20
	No	83	97	180
Total		100	100	200

Odds ratio = ad/bc

= 6.62 (95% CI, 1.88 – 23.39)

Mantel Haenszel Chi –square = 10.834, p value = 0.001

TABLE 6(B)

Parameter Estimates

Hypertension[a]		B	Std. Error	Wald	df	Sig.	Exp(B)	95% Confidence Interval for Exp(B)	
								Lower Bound	Upper Bound
1	Intercept	-4.625	1.018	20.630	1	.000			
	BMI	.187	.041	20.752	1	.000	1.206	1.113	1.307
	[Lepgenecode=]	0[b]	.	.	0	.	.	.	.

a. The reference category is: 2.

b. This parameter is set to zero because it is redundant.

Odds ratio after adjusting BMI = 1.206 (95% CI, 1.113 – 1.307)
P = 0.000

TABLE – 7

ONE WAY ANOVA TO COMPARE THE MEAN VALUE BETWEEN GENO TYPES

Variables		N	Mean	Std. Dev	Min	Max	P-Value
Leptin	II/II	41	28.59	33.20	0.70	131.50	0.883
	I/II	35	25.10	28.36	0.71	99.90	
	I/I	14	25.81	33.01	0.94	103.50	
	Total	90	26.80	31.06	0.70	131.50	
Insulin	II/II	41	10.86	18.72	0.01	100.40	0.940
	I/II	35	9.88	14.66	0.07	58.70	
	I/I	14	11.60	16.05	1.03	53.81	
	Total	90	10.60	16.66	0.01	100.40	
HOMA-IR	II/II	41	2.42	3.972	0.00	19.83	0.929
	I/II	35	2.29	3.592	0.01	14.49	
	I/I	14	2.76	4.036	0.19	13.29	
	Total	90	2.42	3.798	0.00	19.83	
BMI	II/II	105	24.84	4.468	17.09	38.54	0.932
	I/II	75	24.75	4.269	17.12	35.18	
	I/I	20	25.16	3.607	20.00	32.39	
	Total	200	24.84	4.297	17.09	38.54	

TABLE – 8

CORRELATION ANALYSIS

		Leptin	HOMA-IR	BMI
Leptin	Correlation		0.269	0.694
	P-Value		0.010	<0.001
	N		90	90
HOMA-IR	Correlation	0.269		0.283
	P-Value	0.010		0.007
	N	90		90
BMI	Correlation	0.694	0.283	
	P-Value	<0.001	0.007	
	N	90	90	

TABLE - 9

CORELATIONS		CASES			CONTROLS		
		PEARSON	SIG. (2-TAILED)	N	PEARSON	SIG. (2-TAILED)	N
LEPTIN LDL	LEPTIN	1		61	1		30
	LDL	.086	.510	61	.196	.299	30
	LEPTIN	.086	.510	61	.196	.299	30
	LDL	1		100	1		100

CORELATIONS		CASES			CONTROLS		
		PEARSON	SIG. (2-TAILED)	N	PEARSON	SIG. (2-TAILED)	N
LEPTIN HDL	LEPTIN	1		61	1		30
	HDL	.247	.055	61	.295	.114	30
	LEPTIN	.247	.055	61	.295	.114	30
	HDL	1		100	1		100

CORELATIONS		CASES			CONTROLS		
		PEARSON	SIG. (2-	N	PEARSON	SIG. (2-	N

			TAILED)			**TAILED)**	
LEPTIN	**LEPTIN**	1		61	1		30
TGL	**TGL**	.039	.764	61	-.392*	.032	30
	LEPTIN	.039	.764	61	-.392*	.032	30
	TGL	1		100	1		100

*. CORRELATION IS SIGNIFICANT AT THE 0.05 LEVEL (2- TAILED)

CORELATIONS		**CASES**			**CONTROLS**		
LEPTIN		**PEARSON**	**SIG. (2-TAILED)**	**N**	**PEARSON**	**SIG. (2-TAILED)**	**N**
	LEPTIN	1		61	1		30
CHOLESTEROL	**CHOLESTEROL**	.163	.209	61	.100	.599	30
	LEPTIN	.163	.209	61	.100	.599	30
	CHOLESTEROL	1		100	1		100

**. CORRELATION IS SIGNIFICANT AT THE 0.01 LEVEL (2- TAILED)

TABLE – 10

REGRESSION ANALYSIS FOR LEPTIN

MODEL SUMMARY

Model	R	R Square	Adjusted R Square
1	0.845[a]	0.714	0.693

Predictors	Regression Co-Efficient (Beta)	Std. Error (Beta)	95.0% CI for Beta		P-Value
			Lower Bound	Upper Bound	
(Constant)	-150.923	16.537	-183.814	-118.032	
Age	0.504	0.258	-0.008	1.017	0.054
Gender	35.027	4.807	25.466	44.588	<0.001
BMI	3.956	0.455	3.050	4.862	<0.001
Smoking	7.503	5.076	-2.593	17.599	0.143
Alcohol	2.427	5.255	-8.024	12.878	0.645
LEP GENOTYPE	2.092	2.608	-3.096	7.279	0.425

TABLE - 11

MULTIPLE LOGISTIC REGRESSION ANALYSIS FOR CASES (HTN)

Factors		Adj OR	95% CI for OR		P-Value
			LL	UL	
Age		1.052	1.004	1.102	0.034
Gender	Male	1.000			
	Female	0.792	0.362	1.734	0.559
BMI		1.256	1.141	1.383	<0.001
LEP GENOTYPE	II/II	1.000			
	I/II	2.723	1.364	5.435	0.004
	I/I	13.860	3.310	58.044	<0.001
Smoking	No	1.000			
	Yes	1.306	0.501	3.401	0.585
Alcohol	No	1.000			
	Yes	3.319	1.073	10.267	0.037
TGL		0.998	0.987	1.010	0.772
HDL		0.989	0.890	1.100	0.842
LDL		0.991	0.976	1.006	0.227

TABLE - 12

HARDY-WEINBERG EQUILIBRIUM

Variables		Group				P-Value
		Observed		Expected		
		N	%	N	%	
LEP GENOTYPE	II/II	105	52.5	101.5	50.8	0.709
	I/II	75	37.5	82.0	41.0	
	I/I	20	10.0	16.5	8.2	
Total		200	100	200	100	

RESULTS

The estimated levels of various biochemical parameters namely fasting plasma glucose, serum urea, serum creatinine, plasma triglycerides, plasma total cholesterol, plasma HDL, calculated LDL, fasting serum leptin, fasting serum insulin, HOMA-IR levels along with calculated BMI, exposure to smoking and alcohol all of which are risk factors for essential hypertension were tabulated separately for cases and controls in Master chart no.1 – hypertensive subjects and Master chart no.2 – apparently healthy controls.

Descriptive statistics Tables - (A,B) provide information about the descriptive statistics of age, sex, systolic BP, diastolic BP, BMI, smoking, alcohol, biochemical parameters like fasting plasma glucose, serum urea, serum creatinine, plasma lipid profile, leptin genotype, serum leptin, serum insulin, HOMA-IR.

CASES :

Age : 52.8 ± 6.3 years

Sex : male:51, female:49

BMI:26.3 ± 4 kg/m^2

Systolic BP: 150 ± 11 mm of Hg

Diastolic BP: 96 ± 8 mm of Hg

Controls :

Age :50.4 ± 7.8 years

Sex : male:56, female: 44

BMI:23.36 ± 4 kg/m²

Systolic BP: 115 ± 7 mm of Hg

Diastolic BP: 74 ± 4 mm of Hg

Table 1: shows comparision of the means of various parameters between cases and controls using Chi-Square test. Significant difference (Pvalue <0.001) was observed between the genotype distribution among cases and controls.

Table 2: shows comparision of the means of various parameters between cases and controls using Independent samples t-test. Significant difference were observed many parameters like age,BP,serum creatinine, serum leptin,insulin and HOMA-IR.

Table 3: depicts the distribution of genotypes & their frequencies among hypertensives and apparently healthy controls. The genotype frequencies when both cases and controls were combined were I/I = 20, I/II =75 and II/II = 105. The genotype distribution and allele frequencies were strikingly different among cases and controls. Class I/classI genotype was relatively higher in cases and than controls and had a significant p value (17% against 3 % ; p value = 0.001)

The alleles of the LEP-tet polymorphism in the 3'-UTR of LEP gene consisted of 2 groups of various sized alleles: shorter form (class I) and longer form (class II)[230].

Table 4: depicts the allele frequencies (Class I &Class II) among hypertensives and apparently healthy controls.

Class I allele was more frequent in hypertensives than in controls (39.0% *vs.* 18.5%;P <0.001).

Table 5: shows a significant relation of class I allele to its associated risk of essential hypertension with an odds =2.81657 and a P-value< 0.0001 (Wald 95% CI: 1.785 >2.817> 4.445)

Table 6(A): shows significant association between class I / class I genotype and essential hypertension (Odds ratio = 6.62 (95% CI, 1.88 – 23.39);p value = 0.001).

Table 6(B): This association was still significant with a P value = 0.000 even after adjusting for BMI. Odds ratio after adjusting BMI = 1.206 (95% confidence interval, 1.113 – 1.307).

Table 7: shows One way ANOVA to compare the means of serum leptin,serum insulin, HOMA-IR and BMI between the genotypes. None of the above parameters were statistically significant.

Table 8: shows the determination of correlation between serum leptin levels, HOMA-IR and BMI using Pearson correlation analysis. Serum leptin levels correlated significantly with HOMA-IR and BMI with a P value of 0.010 and 0.001 respectively. Also HOMA-IR and BMI showed a significant correlation with a P value of 0.007.

Table 9 : shows the evaluation of any correlation between serum leptin levels and lipid parameters by Pearson correlation and no significant correlation were found .

Table 10 : shows Multiple linear regression analysis for serum leptin levels with various explanatory variables. Serum leptin levels depends linearly with female sex and BMI with P values of <0.001 in both.

Table 11 : shows Multiple logistic regression analysis for cases with hypertension. It showed that age, BMI, alcohol, Class I/Class I genotype and Class I/Class II(P value = 0.004) genotype had independent risks for hypertension. Also it indicated that Class I/Class I genotype with a P value of <0.001 was a significant and independent predictor of hypertension.

Table 12: shows Hardy-Weinberg principle calculation. The genotype and allele distribution were found to be in Hardy-Weinberg equilibrium with a P value of 0.709.

Discussion

DISCUSSION

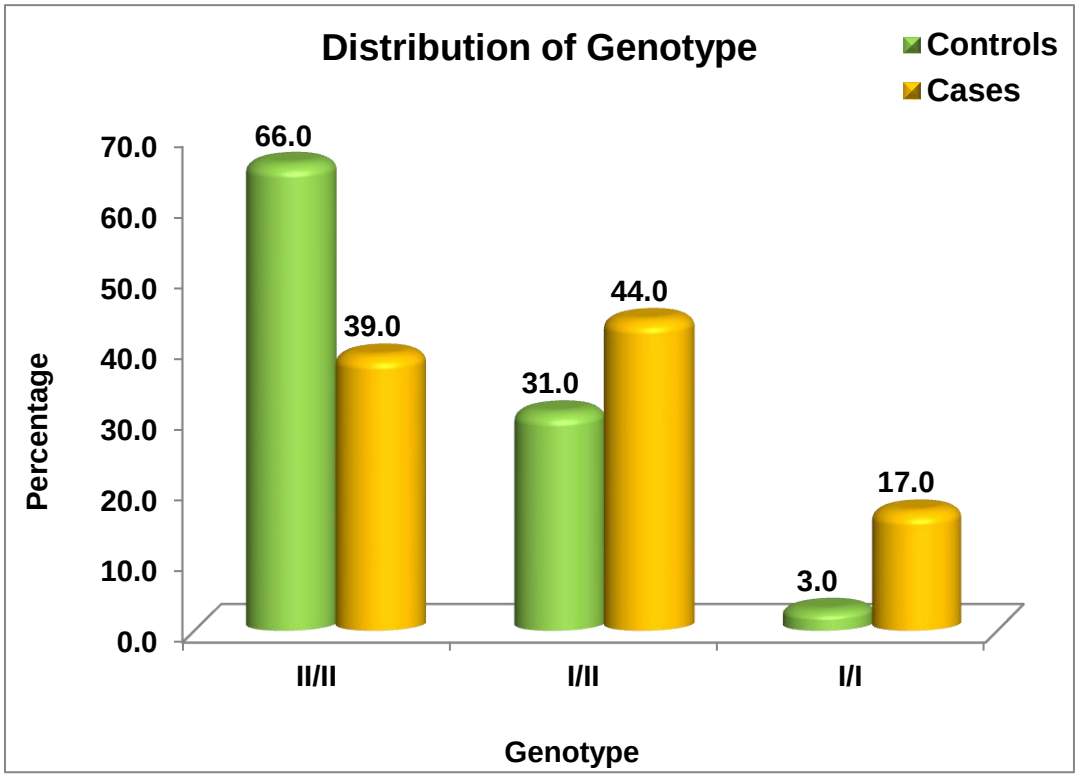
The association of tetranucleotide (TTTC)n repeat polymorphism at 3'-UTR of leptin gene with essential hypertension was examined in this study. The ranges of fasting plasma glucose, serum urea and creatinine, fasting plasma lipid profile including triglycerides (TGL), total cholesterol (TC), HDL-C and LDL-C and serum insulin and leptin among the apparently healthy controls in this study were analysed

Fasting Plasma Glucose	=	88.7 ± 7.6
Serum Urea	=	31.4 ± 4.2
Serum Creatinine	=	0.83 ± 0.16
Plasma Triglycerides	=	110 ± 40.61
Total Cholesterol	=	184.27 ± 23.59
Plasma HDL	=	37.98 ± 4.33
Plasma LDL	=	124.99 ± 23.96
Body Mass Index	=	23.36 ± 4.08
Serum insulin	=	3.35 ± 4.68
Serum leptin(males-BMI<23)	=	2.09 ± 1.45
Serum leptin(females-BMI<23)	=	5.96 ± 3.19
HOMA-IR	=	1.45 ± 2.09

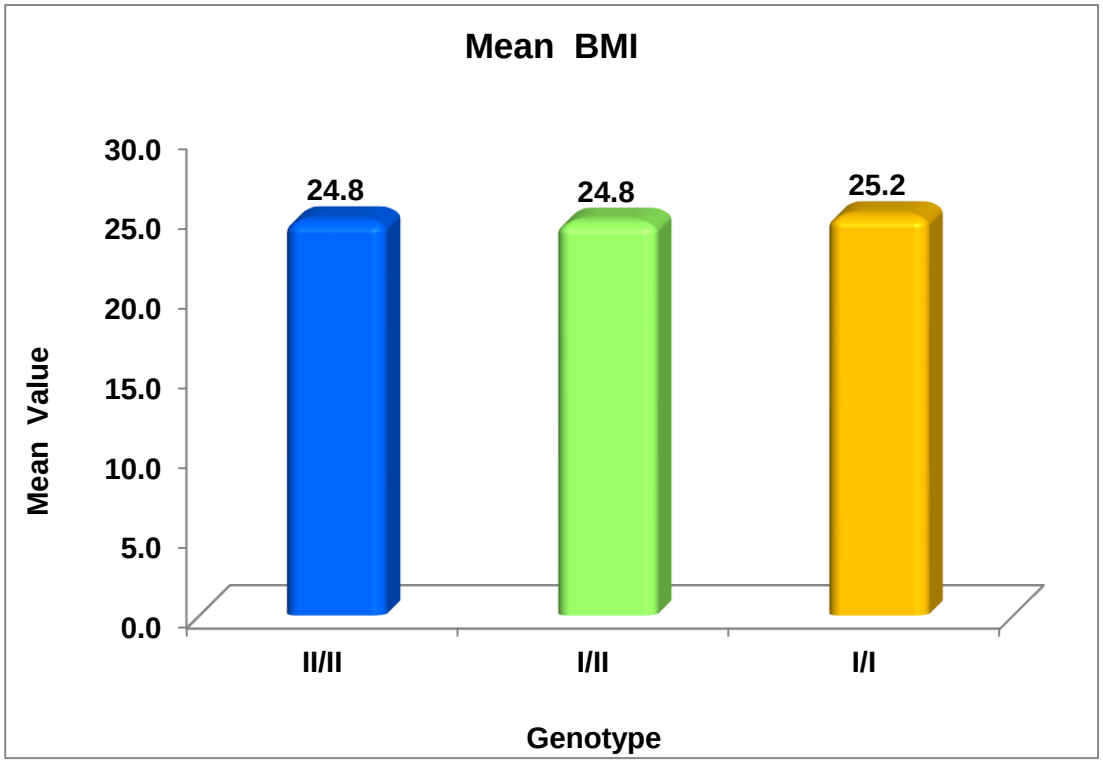
- It is a recognized fact that leptin correlates well with amplified adiposity[57] . This was true in our study. We observed that correlation of leptin concentrations and body mass index in both hypertensives (pearson correlation=0.678,p=0.000) and apparently healthy controls (pearson correlation=0.730,p=0.000) were significant. Thus as expected, positive association was obtained between leptin and measures of increased adiposity.
- The difference between serum leptin levels among hypertensives and apparently healthy controls in our study was statistically significant . This difference in leptin levels in both the hypertensive cases and normotensive controls was in line with many other studies. Leptin levels were reported to be elevated and positively correlated in hypertensives than normotensives in many studies[178,231-236]. In contrast few other studies couldn't obtain a direct positive association between leptin and blood pressure[219,237] .
- Correlation of serum leptin levels and lipid parameters were not significant.
- Serum leptin levels correlated significantly with female gender and BMI. This positive correlation with female gender matched with the data from various studies revealing the sexual dimorphism exhibited by leptin.
- Distribution of class I/class I and class I/classII genotypes were significantly higher in hypertensives than in normotensives. Individuals

with shorter class I allele of LEP-tet polymorphism was found to be significantly associated with hypertension. This was in accordance to the results of similar studies[219,220].

- Class I/Class I genotype and Class I/Class II(P value = 0.004) genotype had independent risks for hypertension. Though Class I/Class I genotype with a P value of <0.001 was a significant and independent predictor of hypertension, the confounding variables like serum leptin, insulin and HOMA-IR could not be excluded because the sample size taken for their estimations were smaller to include them in the multiple logistic regression. This was found to be a limitation to this study.
- The significant association of this tetranucleotide repeat polymorphism with raised blood pressure was not linked to the measure of obesity calculated by body mass index. This was concluded with an odds of 1.206 which was still significant with a P value of 0.000 even after adjusting for BMI. These outcomes put forward the conclusion that though this polymorphism was significantly related to hypertension, this association was not linked to obesity. The above finding supports an animal study where chronic hyperleptinemia directs to a considerable rise in bp independent of obesity[238].


Distribution of Genotype
Controls
Cases
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Percentage
66.0
39.0
31.0
44.0
3.0
17.0
II/II
I/II
I/I
Genotype

-


Mean BMI
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Mean Value
24.8
24.8
25.2
II/II
I/II
I/I
Genotype

-

- Quite a few mechanisms apart from obesity could have been an interlink between this polymorphism and EH. Amongst them are insulin resistance and hyperinsulinemia. Hypertension has been linked to insulin resistance and hyperleptinemia seems to be a cause for IR. Nevertheless IR was not statistically significant among different genotypes. Thus IR is unlikely to be the interlink. Also this polymorphism was not associated with T2DM an insulin-resistant state[230].
- This study also assessed the association of leptin gene polymorphism with hypertension via its effect on the serum leptin level. We didn't find any significant correlation between serum leptin levels and hypertensives among any of the three genotypes. This finding is in line with few other studies[219,223].

Possible mechanisms:

Thus as the phenotypic expression -leptin levels had no significant alterations due to this LEP- tet polymorphism the possible hypotheses to substantiate our observation are as follows.

1. There is a probability that the different alleles of the LEP-tet polymorphism may not have a functional role in determining the concentration of serum leptin levels. However additional studies are needed to prove this hypothesis.

2. Another possibility could be a differential expression of leptin at different sites due to this polymorphism. Leptin expression in nonadipose tissues have been demonstrated by some studies [239,240]. Also studies have revealed a possibility of confined action of leptin via autocrine and paracrine manners. Thus this polymorphism could have resulted in increased expression of leptin at sites responsible for activating the sympathetic nervous system. Such an increased focal non circulating leptin levels could have been accountable for our observation by being responsible for hypertension despite no correlation between serum leptin levels and LEP-tet polymorphism.Additional research works are needed to shed light on this likelihood.

3. Another possibility is the linkage disequilibrium of this polymorphism with some other familiar polymorphisms or mutations either on the coding or regulatory areas of LEP or adjacent genes. Yet, such mutations are very rare in the coding region of leptin gene [241,242]. Additional studies are needed to conclude whether this association is 1° or 2° caused by linkage disequilibrium with some other mutations on the regulatory region of leptin gene or its proximity genes.

Conclusion

CONCLUSION

Differences among the study designs, distributions of hypertension, as well as ethnic and racial differences across study populations may account for the absence of consistency in various other similar studies.

Leptin is thought to produce hypertension by increasing the renal sympathetic activity along with decreased natriuresis[203,204]. Leptin controls the sympathetic nervous system activity in a tissue-specific manner[243].

Though leptin levels were same among both hypertensives and normotensives with raised BMI, this tetranucleotide repeat polymorphism was found to be directly and positively associated with essential hypertension independent of obesity, insulin resistance and hyperinsulinemia.

Usually genetic association studies frequently fall short in reproducing results alike previous research works. To some extent this could be attributed to the polygenetic basis of the disease or to the difference among the investigated populations. The present observations on the genetic background of EH emphasize the call for further research works in determining the complex, polygenetic basis and also gene-gene- and gene-environment interactions or a notable functional testing of the phenotype .

Future Prospects of the Study

SCOPE FOR FUTURE STUDY

- The LEP at chromosome 7q appears as a striking objective for additional researches on linkage analysis.

- Further studies can be done on linkage disequilibrium genetic mapping and its association with essential hypertension.

- With further studies LEP gene could be used as a marker gene for EH.

- LEP and leptin are notable targets for further research works in defining the basis of EH and to elucidate the techniques in the screening, prevention, and treatment of EH.

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ABBREVIATION

ACE	-	Angiotensin Converting Enzyme
AgRP	-	Agouti-Related Protein
AMPK	-	AMP-activated Protein Kinase
ARC	-	Arcuate nucleus
BAT	-	Brown Adipose Tissue
BMI	-	Body Mass Index
BP	-	Blood pressure
CAD	-	Coronary Artery Disease
CART	-	Cocaine-and Amphetamine-Regulated Transcript
CHF	-	Congestive Heart Failure
CHOL	–	Cholesterol
CKD	-	Chronic Kidney Disease
CNS	-	Central Nervous System
CRF	-	Corticotropin Releasing Factor
CVD	-	Cardio Vascular Disorders
DASH	-	Dietary Approaches to Stop Hypertension
DBP	-	Diastolic Blood Pressure
DM	–	Diabetes Mellitus
DMH	-	Dorso-Medial Hypothalamus
DNA	-	Deoxyribo Nucleic Acid
ECF	-	Extra Cellular Fluid
EDHF	-	Endothelium Derived Hyperpolarizing Factor
EDTA	–	Ethylene Diamine Tetra Acetic Acid
EH	-	Essential Hypertension
ESRD	-	End Stage Renal Disease
GLDH	-	Glutamate Dehydrogenase
HDL	–	High Density Lipoprotein
HOMA-IR	-	Homeostatic Model Assessment –Insulin Resistance
HRP	-	Horse Radish Peroxidase
HT	-	Hypertension
IGF	-	Insulin like Growth Factor
IRS2	-	Insulin Receptor Substrate 2
JAK	-	Janus Kinase

JNC	-	Joint National Committee
LEP	-	Leptin gene
LEP-tet	-	Leptin gene tetranucleotide repeat polymorphism
LDL	–	Low Density Lipoprotein
LR	-	Leptin Receptor
LVH	-	Left Ventricular Hypertrophy
MAPK	-	Mitogen-Activated Protein Kinase
MCR	-	MelanoCortin Receptors
MSH	-	Melanoctye Stimulating Hormone
NIDDM	-	Non Insulin Dependent Diabetes Mellitus
NO	-	Nitric Oxide
NPY	-	NeuroPeptide Y
NTS	-	Nucleus Tractus Solitarius
OCP	-	Oral Contraceptive Pills
PAD	-	Peripheral Arterial Disease
PCOS	-	Poly Cystic Ovarian Syndrome
PI3K	-	Phospho-Inositide-3-Kinase
POMC	-	Pro-Opio MelanoCortin
PTB1b	-	Protein Tyrosine Phosphatase 1b
PVN	-	Para-Ventricular Nucleus
QTL	-	Quantitative Trait Loci
RAAS	-	Renin Angiotensin Aldosterone System
RSNA	-	Renal Sympathetic Nervous Activity
SBP	-	Systolic Blood Pressure
SNS	-	Sympathetic Nervous System
STAT	-	Signal Transducer and Activator of Transcription
SOCS-3	-	Suppressors Of Cytokine Signaling protein
TGL	–	Triglyceride
TMB	-	Tetra Methyl Benzidine
Tyr	-	Tyrosine
UTR	-	Un-Translated Region
VLDL	–	Very Low Density Lipoprotein
VMH	-	Vento-Medial Hypothalamus

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone :044 25305301
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CERTIFICATE OF APPROVAL

To
Dr. M. Subarathi
PG in MD Biochemistry
Madras Medical College, Chennai-3,

Dear Dr. M. Subarathi

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled " Association of leptin gene polymorphism in essential hypertension" No. 23032011.

The Following Members of Ethics committee were present in the Meeting held on 17.03.2011 conducted at Madras Medical College, Chennai -3

1. Prof. S.K. Rajan MD	– Chairperson
2. Prof. V. Kangasabai .MD Dean, Madras Medical College, Chennai -3	– Deputy Chairman
3. Prof. A. Sundaram. MD Vice Principal, Madras Medical College, Chennai -3	– Member Secretary
4. Prof. R. Nandhini MD Director, Institute of Pharmacology, MMC, Ch-3	– Member
5. Prof. C. Rajendiran MD Director , Institute of Internal Medicine, MMC, Ch-3	– Member
6. Prof. Geetha Subramanian MD. DM Prof. & Head, Dept, of cardiology, MMC, Ch-3	– Member
7. Prof.. Mohammed Ali MD DM Prof & Head, Dept. of MGE, MMC, Ch-3	– Member
8. Thiru . A. Ulaganathan Administrative Officer, MMC, Ch-3	– Layperson
9. Thiru. S. Govindasamy BA BL	– Lawyer
10. Tmt. Arnold Saulina	– Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/ chairman & Other Members

The Institutional Ethics committee expects to be informed about the progress of the study and SAE occurring in the course of the study , any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

PATIENT CONSENT FORM

Title of the study **:** ASSOCIATION OF LEPTIN GENE POLYMORPHISM IN ESSENTIAL HYPERTENSION**"**

Name : Date :

Age : OP No :

Sex : Project Patient No :

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study.

Signature

PROFORMA

NAME :

AGE :

SEX :

OP.NO:

C/O:

PAST HISTORY:

DM:

DISORDERS OF CVS, THYROID, NEUROLOGICAL, RENAL, ADRENAL, HEPATIC, GI

OBSTUCTIVE SLEEP APNEA:

RECENT INFECTION, INFLAMMATION, LIPID LOWERING DRUGS, NSAIDS, HRT(1 MONTH)

NEUROFIBROMATOSIS, SCLERODERMA

ERSONAL HISTORY: SMOKING ALCOHOL

MENSTRUAL HISTORY:

FAMILY HISTORY:

TREATMENT HISTORY:

BP: / mmHg PR: /min

WT: KGS HT: CMS BMI:

INVESTIGATION REPORTS:

LAB RESULTS:

ECHO:

TO BE MEASURED:

PLASMA GLUCOSE:

BLOOD UREA :

S.CREATININE :

TOTAL CHOLESTEROL:

TGL :

HDL :

LEPTIN :

INSULIN :

PCR RESULTS :

ஆராய்ச்சி தகவல் தாள்.

தங்களது இரத்தம் இங்கு பெற்றுக் கொள்ளப்பட்டது.

சென்னை அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளிடம்
இருக்கும் உயர் இரத்த அழுத்த நோயில் லெப்டின் மரபணுவின்

வேறுபாடை அறிய மேற்கொள்ளப்படும் ஆராய்ச்சி இங்கு நடைபெற்று வருகின்றது.

உயர் இரத்த அழுத்த நோயின் காரணங்கள் பல உள்ளன. அவற்றுள் ஒரு மரபணுவின் வேறுபாடு ஒரு காரணமாக இருக்கலாம் என்பதைக் கண்டுபிடிப்பதே இவ்வாரய்ச்சியின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்பிகிறோம் .இந்த ஆராய்ச்சியில் உங்களுடைய இரத்தத்தை எடுத்து சில சிறப்புப் பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு ஏற்படாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்

. இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது.மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துகொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி :

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு: உயர் இரத்த அழுத்தம் உள்ள நோயாளிகளுக்கு லெப்டின் மரபணுவின் வேறுபாடு உள்ளதா என்பதை கண்டறிதல்

பெயர் : தேதி :

வயது : புற நோயாளி எண் :

பால் : ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

எனக்கு இரத்த பரிசோதனை செய்து கொள்ள சம்மதம்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்.

நான் உயர் இரத்த அழுத்த நோயில் லெப்டின் மரபணுவின்

வேறுபாடை அறிய மேற்கொள்ளப்படும் இந்த ஆராய்ச்சியின் விபரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்து கொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன் .

கையொப்பம்